Improving waterlogging tolerance in barley with molecular and physiological markers

By

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Declaration of Originality

The thesis contains no material which has been accepted for the award of any other degree or diploma by the University or any other institution, and to the best of my knowledge and belief, no material previously published or written by any other person except where due acknowledgement is made in the text of the thesis.

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Publications

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Statement of Co-authorship

The thesis was completed during the course of my enrolment in a PhD degree in the School of Land and Food at The University of Tasmania. The thesis contains no experimental results that have previously presented for any degree at this or other institutions.

The thesis contains one literature review chapter and four research chapters. The literature review chapter (Chapter 2) has been published as a book chapter. Results described in the four research chapters have been or will be published in different journals.

The following people and institutions contributed to the published work undertaken as part of the thesis:

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Table of Contents

Abstract

Over 17 million km^2 of land surface is affected by flooding every year, resulting in severe damages to plants and yield losses in agricultural production around the globe. While the importance of plant breeding for waterlogging tolerance has long been on the agenda, the progress in the field is handicapped by the physiological and genetic complexity of this trait.

The main feature of waterlogged soils is oxygen deprivation, due to slow gas diffusion in water. Decreased oxygen content in waterlogged soils leads to oxygen deficiency, resulting in reduced energy availability for plants. Plant adaptation to waterlogged conditions requires a set of morphological and physiological/biochemical changes. The formation of aerenchyma is one of the most crucial adaptive traits for waterlogging tolerance in wetland species such as rice. Enzymatic scavenging may also contribute to waterlogging tolerance by providing detoxification of reactive oxygen species (ROS). In this thesis, the changes of root porosity (an indicator of aerenchyma formation in roots) and activities in leaves of four major antioxidant enzymes was reported, in six barley genotypes contrasting in waterlogging tolerance. Soil waterlogging caused significant increases in adventitious root porosity in all genotypes. Waterlogging tolerant genotypes showed not only significantly higher adventitious root porosity than sensitive genotypes ($P < 0.01$) but also much faster development of aerenchyma. In contrast, antioxidant enzyme activities in leaves did not correlate with waterlogging tolerance.

Quantifying aerenchyma formation after 7 days of waterlogging can be a fast and reliable approach for the selection of waterlogging tolerant barley genotypes, which is supported by measurements of redox potential (an indicator of anaerobic conditions). This protocol was also used to identify quantitative trait loci (QTL) in a doubled haploid population of barley from the cross between Yerong (tolerant) and Franklin (sensitive) genotypes. The QTL for aerenchyma formation and root porosity were at the same location as one of the major QTL for waterlogging tolerance. The major QTL for aerenchyma formation after 7 days waterlogging treatment on chromosome 4H explained 42.8% of the phenotypic variance. Seven new markers were developed and added onto this region on chromosome 4H. These markers can be effectively used in marker assisted selection to improve waterlogging tolerance in barley.

A wild barley genotype TAM407227 showed very good tolerance to waterlogging and, therefore, provides a useful resource for breeding waterlogging tolerant barley. A high density linkage map was constructed between the wild barley and a cultivated barley Franklin (waterlogging sensitive) using 163 doubled haploid lines. A total of 17 QTL were detected for various traits under waterlogging and control conditions. A new major allele for waterlogging tolerance and aerenchyma formation under waterlogging conditions was identified. The QTL for aerenchyma formation on chromosome 4H explained 76.8% phenotypic variance with a LOD value of 51.4. The high density linkage maps and the QTL for aerenchyma formation can be effectively used for further fine mapping, QTL positional cloning, and marker assisted selection.

Breeding for abiotic stress tolerant crops has drawn increased attention and a large number of QTL for drought, salinity, and waterlogging tolerance in barley have been detected. However, very few QTL have been successfully used in marker assisted selection in breeding programs. We summarized 632 QTL for drought, salinity and waterlogging tolerance in barley. Among all these QTL, 195 major QTL with a LOD value above 3.0 were used to conduct meta-analysis to refine QTL positions for use in marker assisted selection. Meta-analysis was used to map the summarized major QTL for drought, salinity, and waterlogging tolerance from different mapping populations onto the barley physical map. The positions of identified meta-QTL (MQTL) were used to search for candidate genes for drought, salinity, and waterlogging tolerance in barley. Two meta-QTL, MQTL3H.4 and MQTL6H.2, were found to be associated with drought tolerance. Fine mapped QTL for salinity tolerance, HvNax4 and HvNax3, were validated on MQTL1H.4 and MQTL7H.2, respectively. MQTL2H.1 and MQTL5H.3 are also the target regions for improving salinity tolerance in barley. MQTL4H.4 with a fine mapped QTL for aerenchyma formation under waterlogging conditions is the main region controlling waterlogging tolerance in barley. Detected and refined MQTL and candidate genes are crucial for future successful marker assisted selection in barley breeding.

Chapter 1: General Introduction

Over 17 million km^2 of land surface is affected by flooding every year, double the size of the USA (Voesenek and Sasidharan 2013). This results in an estimated annual damage exceeding 60-billion euro (www.dartmouth.edu/~floods/ Archives/2005sum.htm). Crop losses due to excess water are second only to drought, and yield reductions as high as 80 % have been recorded in waterlogged soils (Shaw et al. 2013). Waterlogging is common in duplex, or texture contrast, soils (Setter and Waters 2003). Such soils are widespread in the world, covering ~20 % of the landscape in Australia, Eastern Europe and the Russian Federation (Shaw et al. 2013) and 16 % of soils in the USA (Setter and Waters 2003). Because of this, the production of crop plants that can combine high grain yield with an increased flooding tolerance has been an important objective for decades (Agarwal and Grover 2006).

Under waterlogging, gas diffusion is 10,000 fold slower in solution than in air (Armstrong 1979), thus the depletion of O_2 is a major feature of flooded sites, which creates hypoxia or anoxia around plant tissues. This leads to acute energy crises and very substantial alterations in cell metabolism (and associated yield penalties).

The formation of aerenchyma is one of the alterations maintaining adequate oxygen supply by a series of anatomical and morphological alterations in the root (Perata, Armstrong and Voesenek 2011). Species with higher root porosity are more tolerant to soil flooding, and in many wetland plants, aerenchyma is well developed even in drained conditions (and can be further enhanced in waterlogged conditions), while dry land species often do not form aerenchyma at all (Colmer 2003a). In mature zone of rice (tolerant species) roots, aerenchyma comprised about 45% of the root volume (Colmer 2003a), in stark contrast to only 3% of aerenchyma in the seminal roots of (intolerant) wheat species (Mcdonald, Galwey and Colmer 2001). Another possibility is to minimise radial oxygen losses (ROL) by the formation of a tight barrier in the root peripheral cell layers exterior to the aerenchyma (Mcdonald et al. 2001, Visser et al. 2000). As a result of suberisation and/or lignification of the cell walls, such ROL will facilitate a longitudinal diffusion of O_2 towards the root apex (Nishiuchi et al. 2012), the most metabolically active part of the root. In layman's terms, this is a "business as usual" option that would be the most preferred but that is rather difficult to achieve in full. Nevertheless, in anatomically adapted rice roots, anoxia induced decrease in

the rate of ATP synthesis was only 25 %, in marked contrast to the tenfold decrease in maize (highly sensitive to flooding) root tips (Ratcliffe 1997).

Economisation of ATP consumption includes shutting down energy demanding processes, such as protein synthesis (Branco-Price et al. 2008), and redirecting available ATP resources towards the production of molecular chaperones (e.g. heat shock proteins; (Banti et al. 2010)). This option can be classified as a "survival" strategy, as plant growth will be severely affected, with major implications for yield. Therefore, it is highly unlikely that this option may be sustainable for adaptation to prolonged soil flooding.

Developing a capacity to generate ATP without oxygen is energy efficient sucrose catabolism through sucrose synthase, the preferential use of PPi-dependent enzymes and constrained catabolism of storage compounds such as starch, lipids and proteins (Bailey-Serres et al. 2012a, Licausi 2011). The above complexity of adaptive strategies makes it highly unlikely that one specific "key gene" responsible for waterlogging tolerance could be found and then introduced in high yielding varieties, by either genetic or classical breeding methods. More likely, a thorough pyramiding of suitable traits should be envisaged.

In addition to reduced oxygen availability and associated metabolic shifts, plants grown in flooded soils are exposed to the range of elemental- (Mn, Fe, etc.) and phyto-toxicities (Shabala 2011). Surprisingly, up to now, the focus of plant breeders was predominantly on detrimental effects of anoxia, targeting traits dealing with oxygen uptake and redistribution within plant tissues, while tolerance to these toxicities was essentially neglected.

Based on these severe problems of waterlogging, the development of waterlogging tolerant varieties can be an effective and economical approach to improve production. It is essential to understand the desirable physiological traits of waterlogging tolerant or susceptible plants. Germplasm screening can provide insight into the genetic variation of these desirable traits. Selected genotypes can be further used to improve plant performance in waterlogged environments with breeding and genetic knowledge. Molecular markers have provided plant breeders with a method to improve selection process, and then accelerate breeding programs.

The current work was to identify molecular and physiological markers to improve waterlogging tolerance in barley. By doing this the following objectives were addressed:

- Measuring activities of reactive oxygen species scavenging enzymes in waterlogged plants
- Identifying the linkage between aerenchyma formation under waterlogging stress and waterlogging tolerance of barley
- Mapping QTL controlling aerenchyma formation under waterlogging stress in barley
- Fine mapping QTL controlling aerenchyma formation under waterlogging stress in barley
- Refining QTL for abiotic stresses tolerance in barley for use in barley breeding programs

Chapter 2: Literature review

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This chapter has been published as a book chapter: **Zhang X**, Huang X, Zhou M, Shabala L, Koutoulis A, Shabala S (2015) Plant Breeding for Flood Tolerance: Advances and Limitations. *Genetic Manipulation in Plants for Mitigation of Climate Change*. Springer India, 2015. 43-72.

Chapter 3: Waterlogging tolerance in barley is associated with faster aerenchyma formation in adventitious roots

Abstract

Plant adaptation to waterlogged conditions requires a set of morphological and physiological/biochemical changes. The formation of aerenchyma is one of the most crucial adaptive traits for waterlogging tolerance. Enzymatic scavenging may also contribute to waterlogging tolerance by providing detoxification of reactive oxygen species (ROS). We evaluated the changes of root porosity (as an indicator of aerenchyma formation) and activities in leaves of four major antioxidant enzymes, in six barley genotypes contrasting in waterlogging tolerance. Soil waterlogging caused significant increases in adventitious root porosity in all genotypes. Waterlogging tolerant genotypes showed not only significantly higher adventitious root porosity than sensitive genotypes but also much faster development of aerenchyma. In contrast, antioxidant enzyme activities in leaves did not correlate with waterlogging tolerance. The greatest difference in adventitious root porosity among genotypes was observed after 7 days of waterlogging treatment. This protocol is recommended to be used in future studies to identify molecular markers linked to this trait using appropriate mapping populations. A faster formation of aerenchyma in adventitious roots is one of the key factors for waterlogging tolerance in barley.

Introduction

Excess water and poor soil drainage constraints are estimated to adversely affect approximately 10% of the global land area (Setter and Waters 2003). Dramatic floods occur in all continents and result in an estimated annual damage of crops exceeding 60 billion Euros (www.dartmouth.edu/~floods/Archives/2005sum.htm). With the exception of rice, most crops are sensitive to waterlogging and show significant decline in yield when grown in

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flooded soils. Barley (*Hordeum vulgare* L.) is one of the most sensitive crops, with 20-25% yield losses being reported under waterlogging conditions in the field (P de San Celedonio, Abeledo and Miralles 2014, Setter et al. 1999). One of the main factors influencing plant growth under waterlogging conditions is oxygen deprivation of the roots. Oxygen deprivation reduces ATP levels in plants, causing other physiological and biochemical problems (Bailey-Serres and Voesenek 2008, Colmer 2003a). Common symptoms of waterlogging injury include reduced shoot nitrogen content, leaf area, biomass, shoot growth, root growth and chlorophyll content (Malik et al. 2001, Pang et al. 2004, Zhou et al. 1997).

While application of fertilisers to either soil or foliage (Pang et al. 2007b, Zhou et al. 1997) were shown to improve crop growth and yields under waterlogging conditions, development of waterlogging tolerant genotypes is the most effective and economical approach to improve production under stress conditions. However, little progress was made in breeding barley genotypes for waterlogging tolerance due to the low heritability and highly variable waterlogging conditions (Collaku and Harrison 2005, Zhou 2010). Field-based experiments rather than lab-based physiological traits were mostly used to screen waterlogging tolerant genotypes by breeders (Khabaz-Saberi et al. 2005). Because of the complexity of waterlogging tolerance and variation in field conditions, it might not be effective to make direct selection for waterlogging tolerance in the field. Understanding the mechanisms of waterlogging tolerance makes it possible for plant breeders to target individual physiological traits and pyramid different tolerance related traits to generate barley pre-breeding material with enhanced waterlogging tolerance. To achieve this, it is essential to identify physiological traits which are correlated with waterlogging tolerance.

Different mechanisms are involved in plant tolerance to waterlogging stress. High root porosity resulting from the formation of aerenchyma is effective in avoiding adverse effects caused by waterlogging in cereal crops (Setter and Waters 2003), as internal oxygen supply to roots is enhanced (Colmer 2003b). Root porosity, which is the percentage of gas volume per root volume, is widely used as an indicator of aerenchyma formation reviewed by (Colmer 2003b). Aerenchyma provides an internal system of gas-filled spaces to improve the diffusion of oxygen (Armstrong 1979, Evans 2004). In waterlogged plants, oxygen supply in roots depends mainly on the oxygen transportation from shoots through aerenchyma (Armstrong 1979). The increased concentration of oxygen leads to root aerobic respiration,

resulting in increased energy in roots (Drew et al. 1985). There are two main types of aerenchyma: schizogenous and lysigenous (Nishiuchi et al. 2012). Lysigenous aerenchyma is normally induced under hypoxia conditions among many species (Barrett-Lennard 2003). The mechanism of lysigenous aerenchyma formation has been explored in rice and maize (Nishiuchi et al. 2012). The higher degree of aerenchyma formation is the main mechanism contributing to the better waterlogging tolerance in rice than other dryland cereals (Bailey-Serres and Voesenek 2008).

Plant responses to oxygen deprivation also involve the formation of reactive oxygen species (ROS) such as superoxide radical ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$), hydroperoxyl radical ($HO_2^{\bullet}$), hydrogen peroxide (H_2O_2) (Bailey-Serres and Chang 2005, Blokhina, Virolainen and Fagerstedt 2003), which are harmful to cellular metabolism (Shabala et al. 2014). In addition, some ROS are used as signalling molecules in plant adaptive responses to the range of abiotic and biotic stresses (Baxter, Mittler and Suzuki 2014). Waterlogging is not an exception, and considerable evidence is accumulated that ROS production, by either a plasma membrane (PM) NADPH oxidase and/or mitochondria, regulates plant adaptive responses to oxygen deprivation (Bailey-Serres and Chang 2005). To deal with oxidative stress, plants use different enzymatic and non-enzymatic mechanisms to scavenge overproduced ROS. Major antioxidant enzymes include superoxide dismutase (SOD), peroxidise (POD), catalase (CAT) and ascorbate peroxidases (APX). SOD is able to convert O_2 to $O_2^{\bullet -}$, and then $O_2^{\bullet -}$ to H_2O_2, which is a stable molecule. H_2O_2 can be further scavenged to water by APX, POD or CAT. Non-enzymatic antioxidants include ascorbic acid (AA), glutathione, and phenolic compounds (Blokhina et al. 2003).

Most of the physiological traits associated with waterlogging tolerance are not easy to assess by high-throughput methods and hence this limits the ability to utilize these assays/approaches by breeders. In order to effectively pyramid different tolerance-related traits to improve waterlogging tolerance in a breeding program, it is crucial to identify appropriate quantitative trait loci (QTL) for key traits (including aerenchyma and ROS detoxification) and, thus, appropriate molecular markers closely linked to these traits. For this purpose, efficient screening protocols to accurately phenotype these traits have to be developed. In this study, six barley genotypes differing in waterlogging tolerance were used to address differences in hypothesised key traits. We report that waterlogging tolerant

genotypes had significantly higher adventitious root porosity and developed aerenchyma much faster compared with intolerant (sensitive) genotypes. By contrast, antioxidant enzyme activities did not show any clear correlation with waterlogging tolerance. It is suggested that quantifying root porosity after 7 days of waterlogging may be used as an assay to help identify molecular markers linked to aerenchyma development in barley and to fine map the specific loci conferring this important trait for waterlogging tolerance.

Materials and methods

Experiment 1: waterlogging tolerance, aerenchyma formation, adventitious root porosity, and antioxidant enzyme activities of barley in waterlogged brown sodosol soil

Plant genotypes and waterlogging treatment

Six barley (*Hordeum vulgare* L.) genotypes were used in this experiment. This included five cultivated barley genotypes (Yerong, Franklin, YSM1, Naso Nijo and Gairdner), and one wild barley (TAM407227). Seeds were obtained from the Australian Winter Cereal Collection or China through a joint project with Chinese researchers on barley germplasm research. Waterlogging tolerance of the genotypes was evaluated in 50-L round bins filled with a brown sodosol soil as previously described (Zhou 2011). Texture, pH, and electrical conductivity (EC) of brown sodosol soil at different depths are given in Table 3.1.Waterlogging treatment started at a three leaf stage and lasted for six weeks. Each replication was repeated three times in the glasshouse from August to October, 2013.

Table 3.1: Texture, pH, and EC (electrical conductivity) of a brown sodosol soil at different depth from the Cressy Research Station, Tasmania, Australia.

Sampling depth (cm)	Texture	pH	EC
0-16	Fine sandy loam	5.3	0.06
16-27	Fine sandy loam	5.9	0.02
27-60	Heavy clay	6.2	0.06
60-150	Heavy clay	7.2	0.15

Plant growth measurements

After 7 days waterlogging treatment, one plant from each replication was removed. Longest adventitious root length, adventitious root number, shoot dry weights, and root dry weights were measured.

Root porosity

Root porosity of all genotypes was measured at 0 (just before waterlogging treatment), 1, 3, 5, 7, 14, 21, 28, 35, and 42 days after waterlogging treatment. Measurement of root porosity was based on the buoyancy of the adventitious roots before and after vacuum infiltration (Raskin 1983), using equations modified by (Thomson et al. 1990). Adventitious roots of plants were dug out from soils and carefully washed with water. Approximately 0.3 to 0.4 g (fresh weight) of each sample was used for measurements.

Aerenchyma formation

Adventitious roots were sampled from TAM407227, Yerong, Franklin and Naso Nijo at day 0 (before waterlogging treatment) and at day 7 (7 days after waterlogging treatment). About 2 cm long root segments were taken from the mature zone, approximately 6 cm from the root apex. Cross sections were cut by free-hand with razor blades (Pang et al. 2004) and observed under a bright field light microscope (Olympus BX41). Based on digital images (Olympus DP20), root aerenchyma area and total root cross sectional area were measured using the public domain UTHSCSA ImageTOOL program (http://compdent.uthscsa.edu/).

Antioxidant enzyme activities

Antioxidant enzyme activities were measured at 7 and 14 days after waterlogging treatment. Fresh fully expanded green leaves (0.5 g) were sampled and homogenized using a mortar and pestle under chilling conditions with 5 mL of 50 mM phosphate buffer, pH 7.8, containing 0.1 mM EDTA and 2% PVP. The homogenates were centrifuged at 12,000 rpm for 20 min at 4°C. The supernatants were used for enzyme assays with a spectrophotometer (Genesys10S UV-VIS). Protein concentrations in the extracts were measured at 595 nm (Bradford 1976).

Superoxide dismutase (SOD) activity was measured with the photochemical nitroblue tetrazolium (NBT) method (Beyer Jr and Fridovich 1987). The 3 mL reaction mixture contained 2 mL solution A (0.05 M pH 7.8 phosphate buffer with 112.5 µM NBT, 19.5 mM methionine, 0.15 mM EDTA), 0.95 mL solution B (0.05 M pH 7.8 phosphate buffer with 60 µM riboflavin), and 0.05 mL enzyme sample solution (tissue extract). The absorbance was recorded at 560 nm after 10 min reaction in a light incubator. One unit of SOD was defined as the amount of enzyme that inhibited 50% of NBT photo reduction (U mg^{-1} protein).

Catalase (CAT) activity was assayed by the decrease of absorbance at 240 nm resulting from the decomposition of H_2O_2 (Aebi and Packer 1984). The 3.1 mL reaction mixture contained 1.5 mL of 0.2 M phosphate buffer (pH 7.8) including 1% PVP; 1 mL H_2O; 0.4 mL 0.1 M H_2O_2; and 0.2 mL of the tissue extract. One unit of CAT was defined as 0.01 decrease of absorbance at 240 nm per milligram protein per minute (U mg^{-1} protein min^{-1}).

Ascorbate peroxidase (APX) activity was measured by the reduction of absorbance at 290 nm as a consequence of ascorbic acid oxidation induced by enzymes (Nakano and Asada 1981). The 3 mL reaction mixture contained 2.5 mL of 0.05 M phosphate buffer (pH 7.0) including 0.1 mM EDTA; 0.2 mL 5 mM ascorbic acid; 0.2 mL 0.01 M H_2O_2; and 0.1 mL of the tissue extract. One unit of APX was defined as the amount of enzyme that oxidised ascorbic acid per milligram of protein per minute (µmol·mg^{-1} prot·min^{-1}).

Peroxidase (POD) activity was assayed with the increase of absorbance at 470 nm due to the guaiacol oxidation induced by enzymes (Chance and Maehly 1955). The 3.1 mL reaction mixture contained 2.55 mL of 0.1 M phosphate buffer (pH 7.0) including 0.1 mM EDTA; 0.2 mL 1% guriacol; 0.3 mL 0.01 M H_2O_2; and 0.05 mL of the tissue extract. One unit of POD was defined as 0.01 increase of absorbance at 470 nm per milligram protein per minute (U·mg^{-1} prot·min^{-1}).

GABA contents

GABA contents in roots of plants were measured at 7 days after waterlogging treatment described by (Bai et al. 2009). Roots (200 mg) were sampled and homogenised with 3 mL 4 % acetic acid. The homogenate was deposited for 1 h for sufficient extraction of GABA. Three

millilitres of ethanol was further added to the samples, and then centrifuged at 12,000g for 20 min. The supernatant was collected and used for the measurement of GABA content.

Lactic acid contents

Lactic acid contents in roots of plants were measured at 7 days after waterlogging treatment as described by (Xia and Saglio 1992). Roots (200 mg) were sampled and homogenised in 10 % perchloric acid and neutralised with KOH. Samples were centrifuged at 12,000g for 20 min, and the supernatant was collected and used for the measurement of lactic content.

UPLC-MS/MS analysis of lactic acid and GABA

Samples were analysed using a Waters Acquity H-Class UPLC instrument coupled to a Waters Xevo triple quadrupole mass spectrometer. A Waters Acquity UPLC BEH Amide column (2.1 mm×150 mm×1.7 μm) was used. The mobile phase consisted of two solvents: 95 % (v/v) acetonitrile in water with the addition of 0.1 % (v/v) formic acid and 0.075 % (v/v) ammonium hydroxide (solvent A) and 2 % (v/v) acetonitrile in water with the addition of 0.2 % (v/v) formic acid and 0.1 % (v/v) ammonium hydroxide (solvent B). The UPLC program was 100 % solvent A held for 4 min, then to 86 % solvent A: 14 % solvent B at 12 min, held for 0.5 min, and this was followed by re-equilibration to starting conditions for 5 mins. The flow rate was 0.50 mL min^{-1}; the column was held at 60 °C; and the sample compartment was at 6 °C. Lactic acid extracts were analysed as the neat solution, while GABA ex-tracts were diluted 50 times with laboratory water. Injection volume was 2 μL. Approximate retention times were 4.7 min for lactic acid and 11.9 min for GABA.

The mass spectrometer was operated in positive and negative ion electrospray modes with a needle voltage of 2.7 kV. The ion source temperature was 130 °C; the desolvation gas was N_2 at 950 L h^{-1}; the cone gas flow was 100 L h^{-1}; and the desolvation temperature was 400 °C. Quantitative data was collected in selected ion recording (SIR) mode monitoring (m/z) 89.1 $[M-H]^-$ (cone voltage 22 V) for lactic acid and (m/z) 104.1 $[M+H]^+$ (Cone voltage 15 V) for GABA. Analyte identifications were confirmed by simultaneous multiple reaction monitoring (MRM) analysis using the following precursor to product transitions: (m/z) 89.1 $[M-H]^-$ to (m/z) 43.0 $[M-H]^-$ for lactic acid and (m/z) 104.1 $[M+ H]^+$ to (m/z) 87.0 $[M+H]^+$ for GABA. Cone voltages were as described above, with collision energies of 10 V for both analytes.

Quantitation was undertaken by external calibration curves within the ranges 0.25 to 10 μg mL^{-1} (lactic acid) and 0.05 to 1.0 μg mL^{-1} (GABA). Sample matrix suppression was assessed by sample extract spike recovery at 2 μg mL^{-1} (lactic acid) and 0.5 μg mL^{-1} (GABA).

Experiment 2: adventitious root porosity, and antioxidant enzyme activities of barley in aerated commercial potting mixture

As shown in Table 3.1, texture of brown sodosol soil was fine sandy loam to 27 cm depth of soil, and heavy clay from 27 cm to 150 cm depth. The brown sodosol soil was normally waterlogged with slow drainage when observed during the winter growing season at the Cressy Research Station, Tasmania, Australia. This soil also showed poor drainage in the tanks, so an alternative was needed to ensure growth in aerobic root zones. Therefore, in order to maintain the aerobic conditions in a substrate, six barley (*Hordeum vulgare* L.) genotypes, the same as described in Experiment 1, were sown and grown in 50-L bins, filled with a pine bark/loam-based potting mix with premixed slow release fertiliser.

Plant growth measurements

Growth parameters were also recorded when barley plants were grown in aerated potting mix. Longest adventitious root length, adventitious root number, shoot dry weights, and root dry weights were measured 7 days after 3 leaf stage of barley, the same period of time and method described in experiment 1.

Root porosity

Root porosity of all genotypes in aerobic conditions was measured at 7, 14, 21, 28, 35, and 42 days after 3 leaf stage of barley, the same period of time and method described in experiment 1.

Antioxidant enzyme activities

Antioxidant enzyme activities in leaves of plants with roots in aerobic conditions were measured at 7 and 14 days after 3 leaf stage of barley, the same period of time and method described in experiment 1.

GABA and lactic acid contents in roots

GABA lactic acid contents in roots of plants with roots in aerobic conditions were measured at 7 days after three-leaf stage of barley, the same period of time and method described in experiment 1.

Statistical analyses

ANOVA was used to examine the differences of plant growth parameters and root porosity in different genotypes, under aerobic conditions and waterlogging treatment. The student's *t*-test was also used to examine the difference of plant growth parameters and root porosity between aerobic conditions and waterlogging treatment.

Results

Waterlogging tolerance and plant growth of the six genotypes

The waterlogging tolerance of all genotypes used in this study was ranked based on a combined score of plant healthiness using 0 to 10 score system (0 = plant is totally dead; 10 = no visual symptoms of stress) (Zhou 2011) after 9 weeks waterlogging treatment. Genotypes showed significant difference in waterlogging tolerance. TAM407227 (9.5) and Yerong (8.0) were the most tolerant, followed by YSM1 (6.5) and Gairdner (5.0). Franklin (1.5) and Naso Nijo (1.0) were the most waterlogging susceptible genotypes. The difference in two contrasting genotypes after 6 weeks waterlogging is illustrated in Figure 3.1. TAM407227 showed very good tolerance to waterlogging, which kept a high growth rate under waterlogging conditions. The results are consistent with those previously reported that Yerong was tolerant while both Franklin and Naso Nijo were very sensitive (Zhou 2011, Zhou et al. 2012, Zhou et al. 2007).

Figure 3.1: The appearance of two contrasting genotypes after 6 weeks waterlogging: TAM407227 (waterlogging tolerance score = 9.5) and Franklin (waterlogging tolerance score = 1.5)

In aerated conditions, there was no significant difference in root parameters (longest adventitous root length, adventitous root number, and root dry weights) among six genotypes. When plants were grown in aerated potting mix, Naso Nijo had considerably higher shoot dry weight than the other genotypes (Table 3.2).

Table 3.2: Plant agronomical characteristics measured from aerated (potting mix) and waterlogged (brown sodosol soil; 7 days of waterlogging) treatments. The stress as administered when plants were at the three-leaf stage. Values for plants grown in aerated potting mix were the means of two plants in each three replicates. Values for plants grown in waterlogged brown sodosol soil were presented as the percentage of the values for plants grown in aerated potting mix.

	Longest adventitious root length		Adventitious root number		Shoot dry weight		Root dry weight	
Genotypes	Aerated (cm)	Waterlogging (% of aerated)	Aerated (cm)	Waterlogging (% of aerated)	Aerated (g)	Waterlogging (% of aerated)	Aerated (g)	Waterlogging (% of aerated)
TAM407227	24.30	95.75	30.00	58.33	0.09	45.67	0.08	43.12
Yerong	20.33	90.18	20.00	71.35	0.22	55.33	0.09	53.57
YSM1	20.73	65.41	17.50	104.17	0.15	85.13	0.09	43.17
Gairdner	21.50	62.67	32.50	73.08	0.11	94.54	0.08	68.00
Franklin	15.95	82.78	26.25	100.00	0.18	72.61	0.09	51.11
Naso Nijo	19.73	67.65	15.83	126.32	0.40	45.53	0.09	40.94

Waterlogging greatly reduced ($P < 0.05$) longest adventitous root length, shoot dry weights, and root dry weights among all these six genotypes (Table 3.2). After 7 days waterlogging, there was no significant difference in terms of shoot dry weights (0.11 g on average) and root dry weights (0.07 g on average). Relatively waterlogging tolerant genotypes, TAM407227 amd Yerong, had much higher ability of maintaining adventitious roots length than the other four genotyps after 7 days waterlogging (Table 3.2). Waterlogging also had a significant impact on adventitous root number ($P < 0.01$). After 7 days waterlogging, the number of adventitious roots increased in Naso Nijo and YSM1; decreased in Yerong, TAM407227, and Gairdner; did not change at all in Franklin (Table 3.2). None of the measured plant growth parameters was significantly correlated with waterlogging tolerance.

Aerenchyma formation in experiment 1

Before waterlogging treatment (day 0), a small proportion of aerenchyma was found in two waterlogging tolerant genotypes, TAM407227 (1.8 ± 0.1%) (Fig. 3.2A) and Yerong (4.4 ± 0.2%) (Fig. 3.2B), but no aerenchyma was found in the roots of Franklin (Fig. 3.2C) and Naso Nijo (Fig. 3.2D). Seven days after waterlogging treatment, aerenchyma was formed in all genotypes (Figs. 3.1E-H). However, the percentage of aerenchyma differed considerably, with waterlogging tolerant genotypes TAM407227 (20.0 ± 2.2%) and Yerong (11.7 ± 1.5%) (Figs. 3.1E-F) showing a much higher percentage of aerenchyma than waterlogging susceptible genotypes Franklin (2.6 ± 0.2%) and Naso Nijo (4.5 ± 0.6%) (Figs. 3.1G- H).

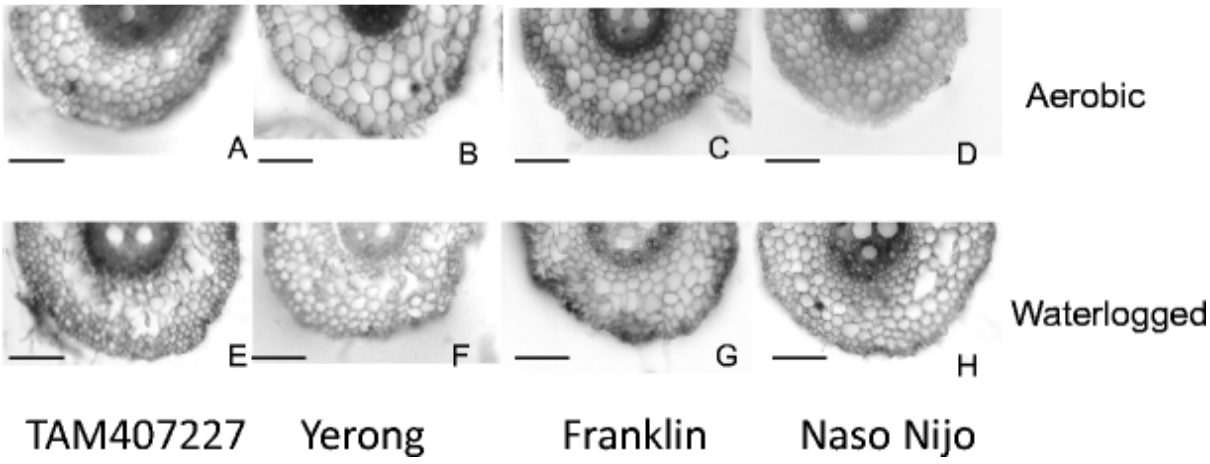

Figure 3.2: Light micrographs of cross section of adventitious roots. Under aerobic conditions (day 0), TAM407227 (A) and Yerong (B) had a small proportion of aerenchyma, while Franklin (C) and Naso Nijo (D) lacked aerenchyma. After 7 days waterlogging, TAM407227 (E) and Yerong (F) had a larger proportion of aerenchyma than Franklin (G) and Naso Nijo (H). Bar = 100 µm.

Root porosity of plants in experiment 1

There was no significant difference in the root porosity on day 0 (before waterlogging treatment) among six genotypes. After waterlogging treatments, root porosity of all the genotypes increased steadily and quickly from day 1 to day 14 (Fig. 3.3). The time of measurements also had a significant influence on the root porosity ($P < 0.01$). On average, the percentage of root porosity increased from about 7.2% at day 0 to 8.1% at day 3, 10.8% at day 7 and 17.7% at day 14. Genotypes showed different responses in root porosity to waterlogging stress. The root porosity of two waterlogging tolerant genotypes, TAM407227 and Yerong increased to approximately 14% at 7 days after waterlogging. In contrast, the most sensitive genotypes, Franklin and Naso Nijo, showed a slower increase of root porosity, with only a slightly higher percentage of root porosity (7% at day 7) than the control (6% at day 0). Both YSM1 and Gairdner showed an intermediate increase in root porosity within the first 7 days of waterlogging treatment, which is consistent with their medium waterlogging tolerance. Significant increases ($P < 0.01$) in root porosity from day 7 to day 14 were found in all six genotypes, from 10.8% to 17.7% on average (Fig. 3.3).

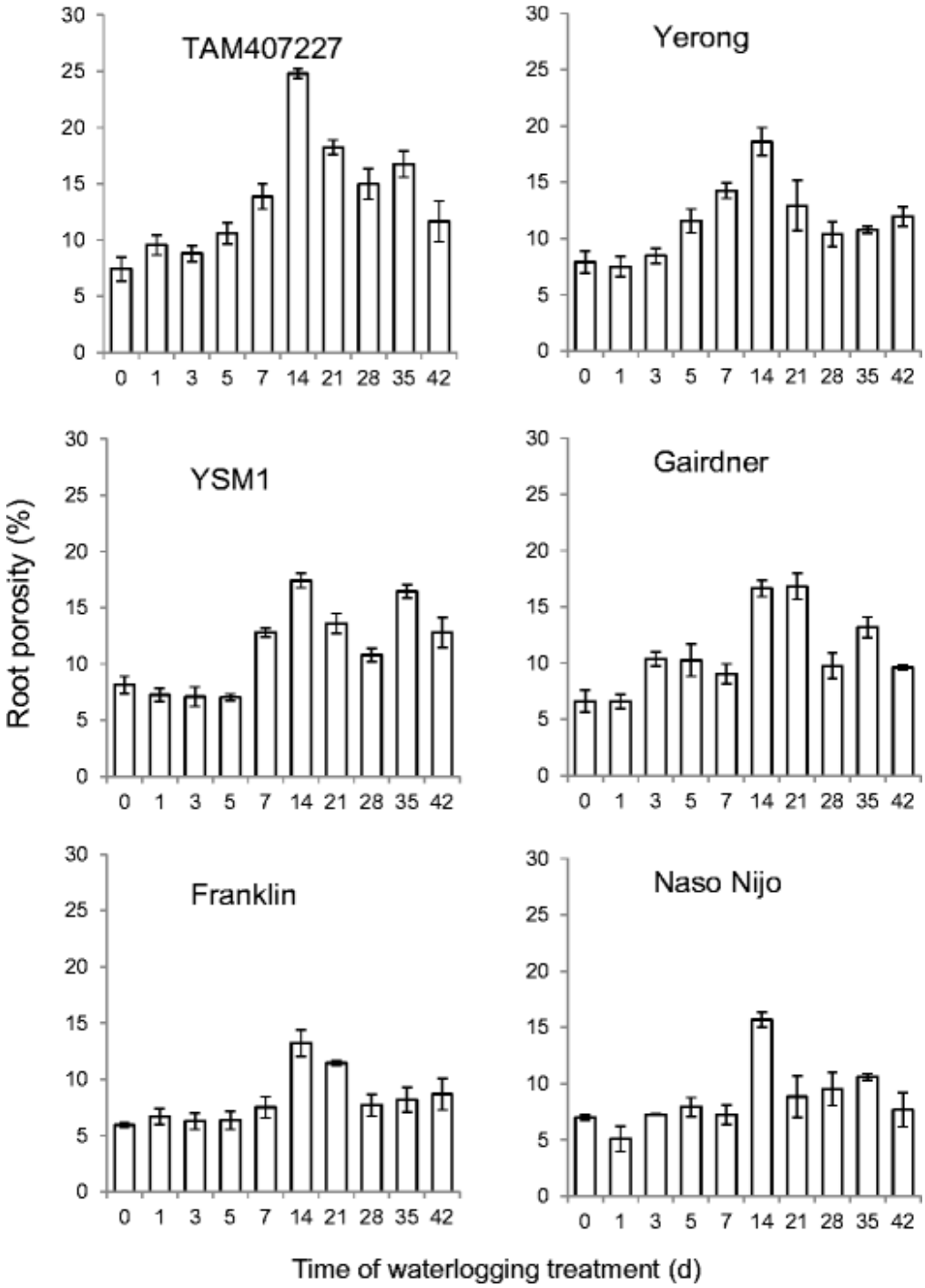

Figure 3.3: Adventitious root porosity of six different barley genotypes after 0, 1, 3, 5, 7, 14, 21, 28, 35, and 42 days of waterlogging in a brown sodosol soil. Waterlogging was started at the three-leaf stage of barley. Values are the means ± standard deviations of three replicates. Each replicate represents roots from three single plants growing in different tanks.

Figure 3.4 compares kinetics of aerenchyma development at early stages of waterlogging stress between contrasting (sensitive—Franklin and Naso Nijo; tolerant—TAM407227 and

Yerong) genotypes. As can be seen, the slope of the curve is drastically different between tolerant (ascending; closed symbols in Fig. 3.4) and sensitive (flat; open symbols in Fig. 3.4) varieties. At later stages, however, the rate of aerenchyma changes is about the same between sensitive and tolerant varieties. Taken together, this suggested that the difference in percentage of aerenchyma comes from the time when the process has started (sooner in tolerant varieties).

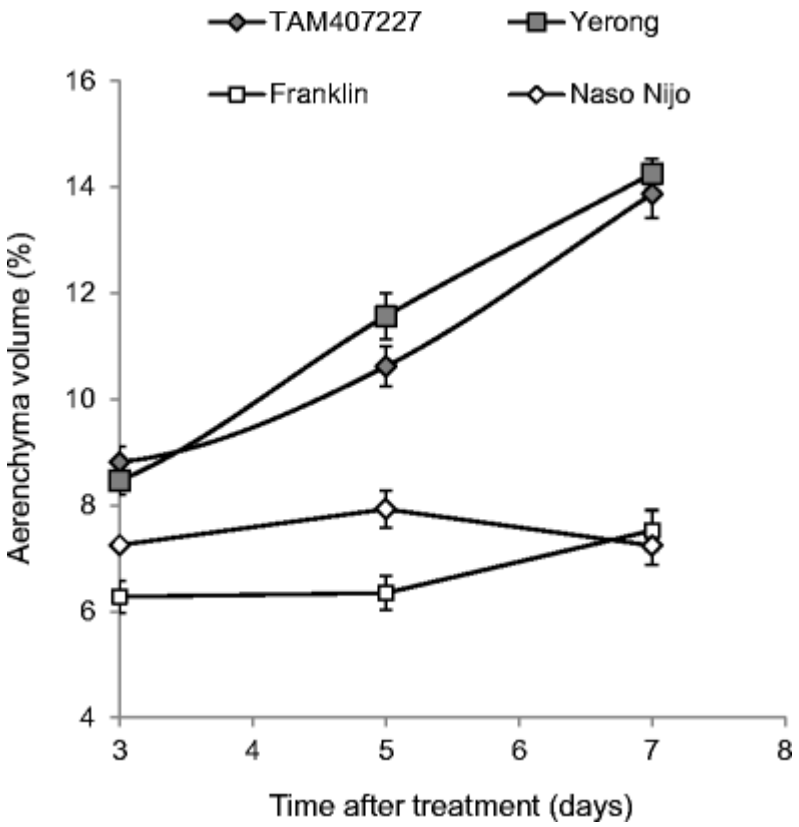

Figure 3.4 Kinetics of aerenchyma development in barley in brown sodosol soil: aerenchyma percentage as a function of time at early stages of waterlogging stress. Mean ± SE (n = 3)

All genotypes showed a decrease in root porosity from day 14 to day 42 (Fig. 3.3). At most stages, waterlogging tolerant genotypes (TAM407227 and Yerong) had higher root porosity than sensitive genotypes (Franklin and Naso Nijo). The biggest differences between tolerant genotypes and sensitive genotypes were found 7 days after waterlogging treatment, with both waterlogging tolerant and intermediate genotypes having a significant increase and waterlogging sensitive genotypes showing a little change in the percentage of root porosity.

Root porosity of plants in experiment 2

When grown in aerobic potting mix, the adventitious root porosity was relatively consistent in a period of six weeks for all the genotypes, ranging from 3-5% (Fig. 3.5), confirming the above observations that no aerenchyma or little aerenchyma was formed when waterlogging stress was not applied. Root porosity for all the genotypes on day 0, in non-waterlogged brown sodosol soil (7.2 ± 0.8%), were significantly higher ($P<0.01$) than the root porosity of all the genotypes in aerated potting mixture (4.0 ± 0.5%). There was no significant difference in root porosity among the selected genotypes when grown in the drained brown sodosol soil before waterlogging started ($P = 0.23$) or when in the aerobic potting mix at the same growth stage ($P = 0.40$).

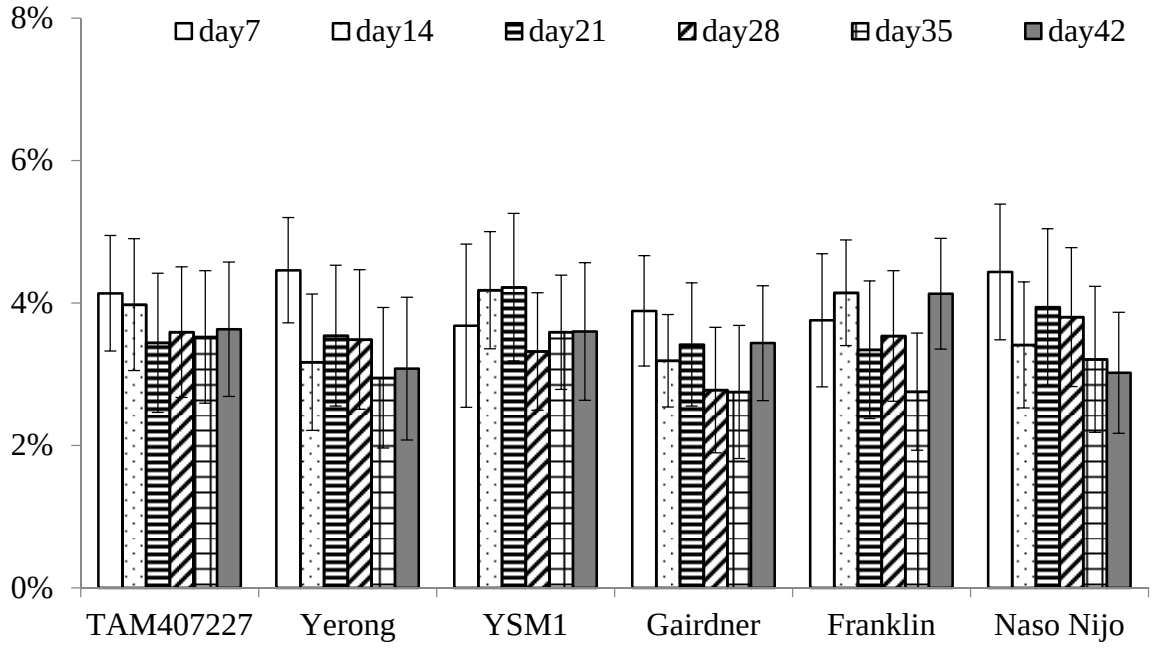

Figure 3.5: Adventitious root porosity of six different barley genotypes in aerobic conditions after 7, 14, 21, 28, 35, 42 days of 3 leaf stage growing barley, the same period of growing stages with measuring adventitious root porosity under waterlogging conditions (Fig. 3.4). Values are the means ± standard deviations of three replicates. Each replicate represents roots from three single plants growing in different tanks.

Antioxidant enzyme activity

Waterlogging stress showed significant effects on antioxidant enzyme activity in leaves (Fig. 3.6A). SOD activity of waterlogging tolerant genotypes TAM407227, Yerong and YSM1 decreased and those of waterlogging sensitive genotypes, Franklin, Naso Nijo and Gairdner increased after 7 days of waterlogging treatment. Except for Gairdner, the 14 days waterlogging treatment showed the opposite trend in the changes of SOD activities, with slight increases in TAM407227 and Yerong, a significant increase in YSM1 but significant decreases in both Franklin and Naso Nijo.

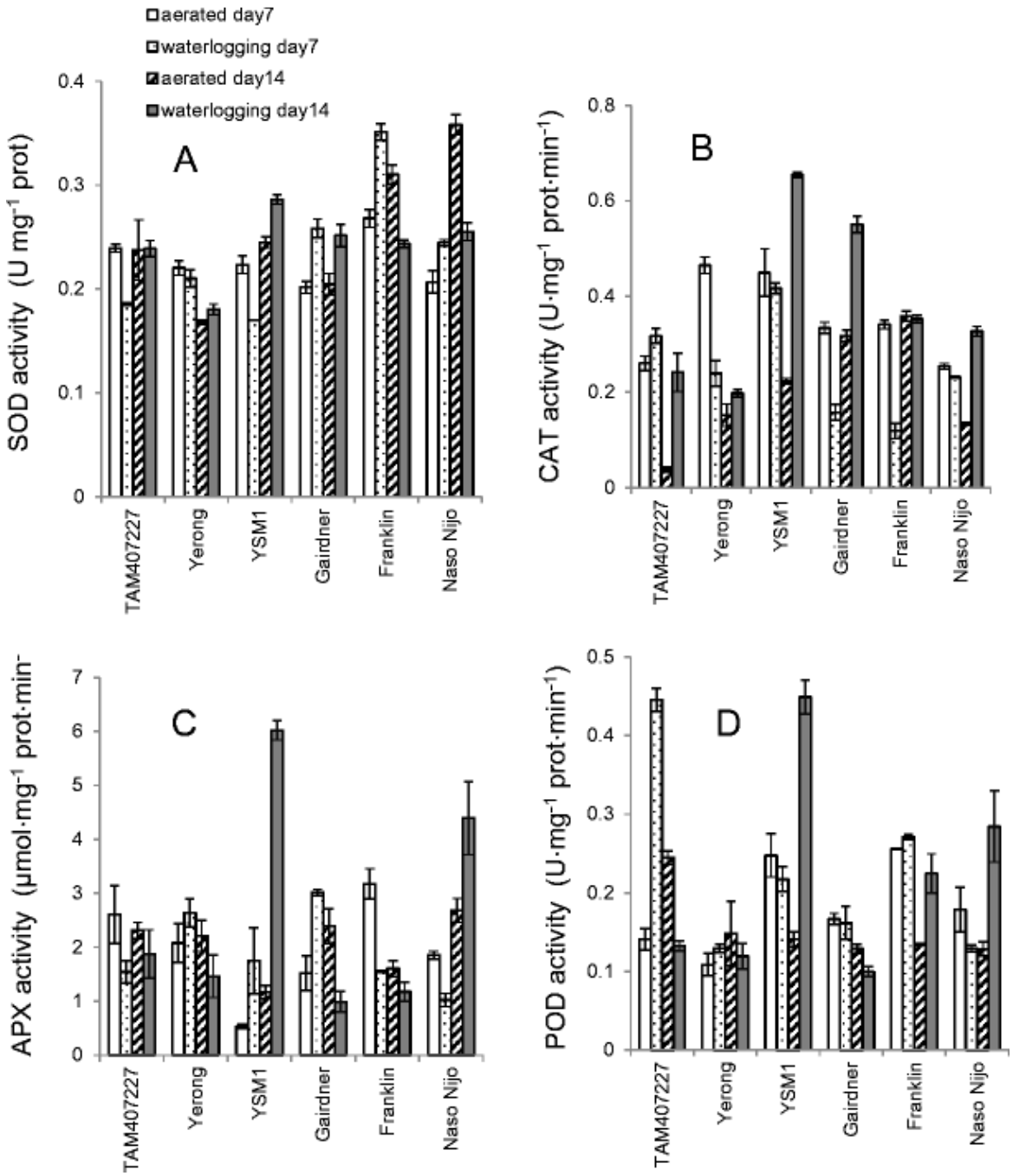

Figure 3.6: Antioxidant enzyme activities, including SOD (A), CAT (B), APX (C), and POD (D), under aerated conditions (experiment 2) and waterlogging stress (experiment 1) after 7 and 14 days waterlogging treatment which started at three-leaf stage of barley. Values are the means ± standard deviations of three replicates. Each replicate represents only green leaves from three single plants growing in different tanks

Seven days of waterlogging treatment caused a significant decrease in CAT activity in leaves of all genotypes except TAM407227 (Fig. 3.6B). Similarly, 14 days waterlogging treatment

changed the pattern of CAT activity. Yerong, YSM1, Gairdner and Naso Nijo showed significant increase in CAT activity, while TAM407227 had a significant decrease in CAT activity after 14 days of waterlogging treatment.

Waterlogging treatment showed no effects on APX activity in leaves of both Yerong and TAM407227, but significantly increased APX activity in YSM1 (Fig. 3.6C). Opposite effects of 7 days and 14 days waterlogging treatments on APX activity were found for Gairdner and Naso Nijo (Fig. 3.6C). The most significant change in APX activity was found in YSM1 with the activity being four times higher than that of control after 14 days waterlogging treatment.

A significant increase in POD activity was only found in leaves of TAM407227 after 7 days of treatment but the activity was much lower than the controls after 14 days of waterlogging (Fig. 3.6D). Waterlogging treatment caused significant increases in POD activity of YSM1, Franklin and Naso Nijo (Fig. 3.6D).

GABA contents in roots

GABA contents in waterlogging-tolerant genotypes (TAM407227 and Yerong) and waterlogging-sensitive genotypes (Franklin and Naso Nijo) increased dramatically after 7-day waterlogging treatment (Fig. 3.7). No significant changes in GABA contents were found in both YSM1 and Gairdner.

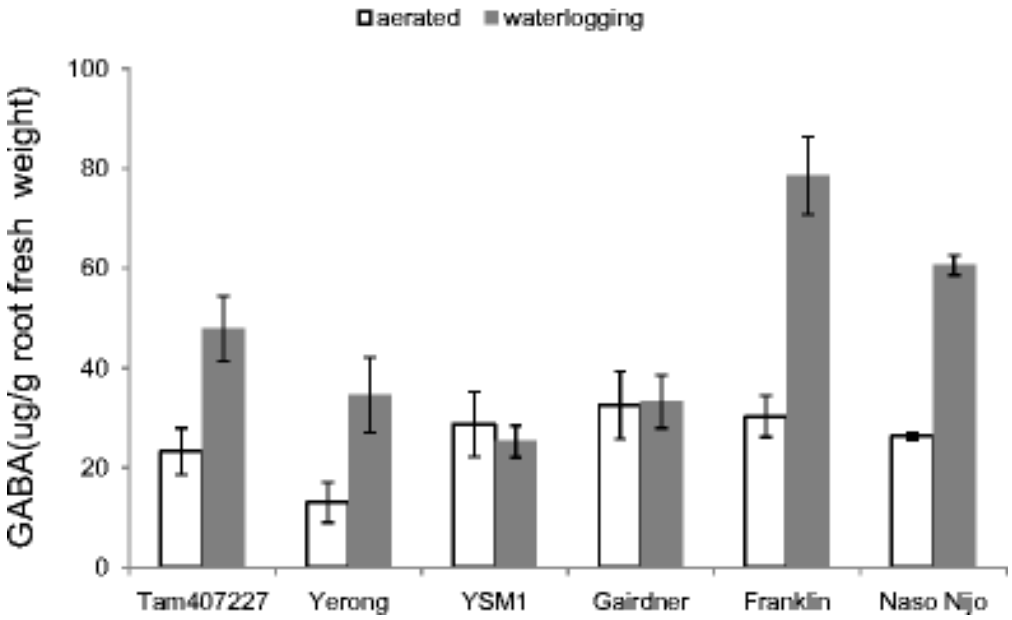

Figure 3.7 GABA contents under aerated conditions (potting mixture, experiment 2) and waterlogging stress (brown sodosol soil, experiment 1) after 7-day waterlogging treatment which started at three-leaf stage. Values are the means ± standard deviations of three replicates. Each replicate represents roots from three single plants growing in different tanks.

Lactic acid contents in roots

Seven days after waterlogging treatment, lactic acid contents in roots increased significantly in YSM1 and Franklin (Fig. 3.8). In contrast, lower lactic acid contents were found in Yerong and Gairdner while lactic acid contents in TAM407227 and Naso Nijo changed little after waterlogging treatment.

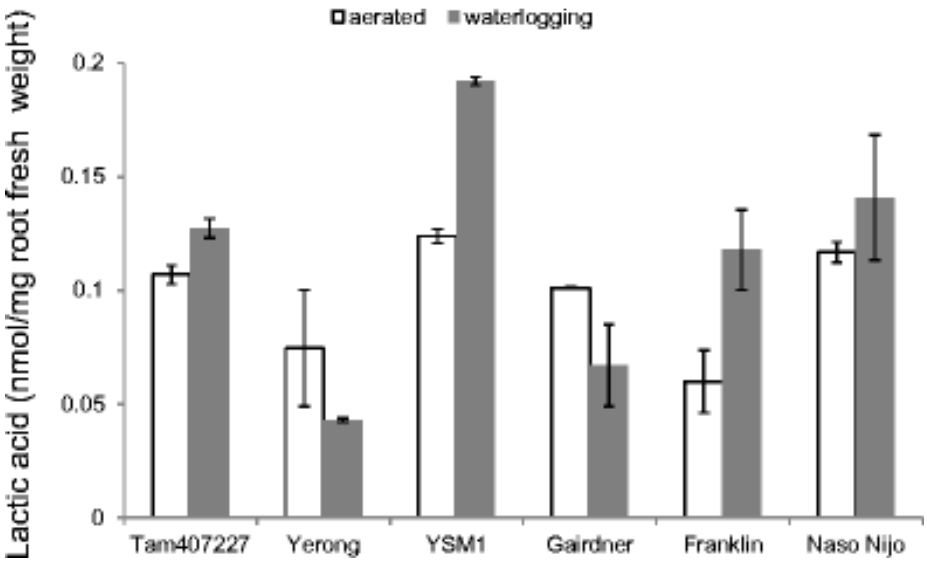

Figure 3.8 Lactic acid contents under aerated conditions (potting mixture, experiment 2) and waterlogging stress (brown sodosol soil, experiment 1) after 7-day waterlogging treatment which started at three-leaf stage. Values are the means ± standard deviations of three replicates. Each replicate represents roots from three single plants growing in different tanks.

Correlations between waterlogging tolerance and physiological traits

The percentage of root porosity at different stages of waterlogging stress showed significant correlations with waterlogging tolerance. The highest correlation between waterlogging tolerance and root porosity was found 7 days after waterlogging treatment (R^2 = 0.91, P < 0.01). Thus, the percentage of root porosity after 7 days of waterlogging treatment can be the best indication for waterlogging tolerance of a variety.

Waterlogging treatment showed significant effects (P < 0.01) on the activity of different antioxidant enzymes in leaves. However, no clear correlation between waterlogging stress tolerance and activity of major enzymatic antioxidants in leaves was observed. The best putative fit was observed between waterlogging tolerance and SOD activities (R^2 = 0.63 after 7 days of waterlogging and R^2 = 0.61 after 14 d of waterlogging). However, none of these correlations were statistically significant (P < 0.05). Moreover, the correlation between SOD activity and waterlogging tolerance was negative, suggesting that, as a very best, elevated SOD levels may be used as stress markers but not as traits conferring waterlogging tolerance in barley.

Discussion

Waterlogging tolerance is associated with faster aerenchyma formation

Oxygen diffusion in water is 10^4 times slower than the diffusion in air (Armstrong 1979). Therefore, roots surrounded by water have very limited oxygen uptake and ATP production is greatly decreased with the oxygen deprivation, resulting in the lack of energy in waterlogged plants (Colmer and Voesenek 2009). Root aerenchyma is a special tissue with gas spaces, forming an internal system to improve the diffusion and thus concentration of oxygen within roots when in waterlogged soil (Armstrong 1979, Colmer 2003a). Increased oxygen concentration in roots leads to higher respiration rates, generating increased energy (ATP) in roots, improving nutrient uptake (Colmer and Greenway 2010) and plant survival under waterlogging conditions (Armstrong and Armstrong 1999, Colmer and Voesenek 2009).

Wetland species, such as rice, are able to form constitutive aerenchyma. The constitutive root porosity in rice can be 20-30%, increasing to more than 40% in waterlogged soils (Colmer 2003a, Steffens et al. 2010). The wild relative of barley *H. marinum* and some wild relatives of maize can also form constitutive root aerenchyma under well-aerated conditions. This ability is expected to be a valuable waterlogging tolerance trait for environments with transient waterlogging since plants with developed aerenchyma adapt to waterlogged soils quickly (Malik et al. 2009, Mano et al. 2008). In our experiments, neither cultivated barley genotypes nor their wild relative TAM407227 formed a significant amount of constitutive aerenchyma in aerobic conditions. All the genotypes had a low percentage of root porosity and no significant differences were found among genotypes. Our results were slightly different from the report by (Broughton et al. 2015), who found not only higher percentage of root porosity but significant differences among barley genotypes when subjected to hydroponic aerated solutions. It is possible that the aerated hydroponic solution generated slight hypoxic conditions (or perhaps increased root ethylene), causing the increase of root porosity in waterlogging tolerant genotypes.

Higher percentage of aerenchyma can also be induced in roots of many plants by waterlogging stress. Waterlogging tolerant species, such as the wild relative of barley *H. marinum* (Garthwaite et al. 2003) have significantly higher root porosity than the susceptible ones under waterlogging conditions. The linkage between root porosity and waterlogging

tolerance were also found in wheat (Mcdonald et al. 2001), maize (Mano and Omori 2013), soybean (Shimamura et al. 2010) and forage legumes (Gibberd et al. 1999, Teakle et al. 2011). In our experiments, waterlogging tolerant barley genotypes had significantly higher root porosity than susceptible genotypes under waterlogging treatment. The tolerant genotypes also had a faster increase of root porosity with accelerated aerenchyma development under waterlogging treatment. In legumes, the faster aerenchyma formation is associated with the recovery of N metabolism in roots (Thomas et al. 2005) and improves the internal oxygen transport from shoot to waterlogged roots, enhancing an increased concentration of oxygen in the root zone (Shimamura et al. 2010, Teakle et al. 2011). The waterlogging tolerant legume *Melilotus siculus* (Teakle et al. 2011) and waterlogging tolerant soybean genotypes (Shimamura et al. 2010, Thomas et al. 2005) were able to form aerenchyma rapidly, reaching more than 20% of porosity after 7 days waterlogging treatment. In contrast, less than 10% of root porosity was detected in the relatively less waterlogging tolerant wheat (Yamauchi et al. 2014a) and canola (Voesenek et al. 1999) after 7 days of waterlogging treatment. In our experiments, two waterlogging tolerant genotypes, TAM407227 and Yerong, started to form aerenchyma within 7 days of waterlogging stress with the root porosity showing a significant increase (from 6% to 14%) at that time. In contrast, two sensitive genotypes, Franklin and Naso Nijo, showed only a slight increase in the percentage of root porosity (from 6% to 7%) at 7 days after waterlogging. Therefore, fast aerenchyma formation is likely a key mechanism in tolerant barley genotypes under waterlogging stress.

The percentage of root porosity of almost all genotypes used in this study reached the highest level at 14 days after waterlogging and declined afterwards. The low and inconsistent root porosity may be caused by the damage to the root system after prolonged waterlogging (more than 14 days). Prolonged waterlogging was reported to induce microelement toxicities, such as Mn^{2+} and Fe^{2+} (Shabala 2011) or toxic secondary metabolites (Pang et al. 2007a). These toxicities affect root nutrient uptake and membrane transport activities (Pang et al. 2007a), resulting in the disturbance of signalling systems in waterlogged plants (Voesenek and Sasidharan 2013) and leading to the damage of the root system.

Waterlogging influence on plants growth

Waterlogging significantly decreased plant growth and development. After 21 days anoxia or waterlogging treatment, growth parameters, including longest adventitious root length, shoot dry weight, and root dry weight were reduced to 20-80% of the same growth parameters in aerated conditions (Broughton et al. 2015, Garthwaite et al. 2003, Mcdonald et al. 2001, Pang et al. 2004), After 7 days waterlogging treatment, longest adventitious root length, shoot dry weight, and root dry weight among all the six genotypes also decreased to 40-95% of the same growth parameters in aerated conditions. Adventitious root number of some *Hordeum* genus and tribe Triticeae crops increased or decreased in anoxia treatment, compared with the adventitious root number of crops in aerated conditions. We had the same result in our experiment. Waterlogging treatment for 7 days might increase the adventitious root number of some barley genotypes, or decrease the adventitious root number in other barley genotypes. But none of the measured growth parameters were significantly correlated with waterlogging tolerance. Therefore, these growth parameters cannot be used as the selection criteria to screen waterlogging tolerance in barley.

Waterlogging tolerance is not related to antioxidant enzyme activities in the leaves

Activities of antioxidant enzymes under waterlogging conditions have been studied in various plant species. Different antioxidant enzyme activities were regarded as the waterlogging tolerance mechanism in different species (Table 3.3). However, there is no widely accepted conclusion as to which enzyme activity can be used as an indicator of waterlogging tolerance since the results on the changes of antioxidant enzyme activities are not consistent as well. Even in the same species, the results vary between different experiments. For example, in maize, SOD, CAT, and APX activity in leaves increased under waterlogging conditions in one experiment (Tang et al. 2010), but decreased under waterlogging conditions in another experiment (Yan et al. 1996). The inconsistency of antioxidant enzyme activities is mainly due to the fact that ROS production and enzyme activities are highly unstable and time-dependent (Fan et al. 2014). In barley leaves, waterlogging stress causing decreased SOD activity in one experiment (Yordanova, Christov and Popova 2004), but increased SOD activity in another experiment (Zhang et al. 2007). Consistent with this, our experiments showed no correlation between different enzyme activities and waterlogging tolerance. Since excessive accumulation of metal ions in plants is a possible factor in triggering ROS

production (Shabala et al. 2014), selecting waterlogging tolerant genotypes can partially be achieved through plant tolerance to toxic Mn^{2+} and Fe^{2+} which often is increased in waterlogged soils (Khabaz-Saberi et al. 2005). This is consistent with our recent study with the demonstration of a significant correlation between Mn^{2+} tolerance and waterlogging tolerance in barley (Huang et al. 2014). In the light of above, we believe that using activity of antioxidant enzyme activities as biochemical markers will not be able to discover QTLs conferring waterlogging stress tolerance in barley.

Table 3.3: Summary of antioxidant enzymes activities under waterlogging conditions in different plant species.

Plant Species	Growing stages	Samples	Waterlogging tolerant mechanims	Increased activities	Decreased activities	Unaffected activities	Reference
Barley	3 leaf stage	Leaves		CAT, APX, and POD	SOD		(Yordanova et al. 2004)
Barley		Leaves	Lower SOD, higher CAT and POD	SOD, CAT, and POD			(Zhang et al. 2007)
Rice	Seedlings	Leaves		CAT	SOD	APX	(Ushimaru et al. 1999)
Rice	14-days-old plants	Roots and shoots	Higher SOD, CAT, and APX	SOD and CAT	APX		(Damanik et al. 2010)
Wheat	Anthesis	Flag leaf	Higher CAT	SOD and CAT			(Sairam and Srivastava 2001)
Wheat	Post anthesis	Flag leaf	Higher POD	POD in tolerant genotypes	SOD and CAT		(Tan et al. 2008)
Maize	2 leaf stage	Leaves	Higher SOD, CAT, and APX	SOD, CAT, and APX	POD		(Tang et al. 2010)
Maize	2 leaf stage	Roots		APX	SOD and CAT	POD	
Tobacco	Seedlings	Leaves	Higher SOD	SOD			(Yu and Rengel 1999)
Lotus	10-days-old plants	Leaves		APX	SOD	CAT	(Ushimaru et al. 2001)
Creeping bentgrass	Mature	Roots	Higher SOD and APX	SOD	APX	POD	(Wang and Jiang 2007)
Citrus	One-year-old-seedlings	Leaves	Higher CAT	SOD and APX	CAT in susceptible genotypes		(Arbona et al. 2008)
Citrus	One year old seedlings	Leaves	Higher SOD and CAT	SOD, CAT, and APX			(Hossain et al. 2009)
Pigeon pea	25-days-old plants	Roots	Higher SOD, CAT, and APX	SOD, CAT, and APX			(Kumutha et al. 2009)
Pigeon pea	25-days-old plants	Roots	Higher SOD, CAT, and APX	SOD, CAT, and APX			(Sairam et al. 2009)

While this work was focused predominantly on aerenchyma and antioxidant enzyme activities, other physiological traits need to be considered in future experiments and, specifically, those important to mitigate damaging effects of energy crisis and cell acidosis under hypoxic conditions. Cytosolic pH decreases sharply in response to anoxia, typically from 7.2 to 6.7–6.8 pH units within minutes or even seconds (Felle 2005, Ratcliffe 1997). This cytosolic pH decrease is believed to represent the optimal conditions for metabolism under suboptimal oxygen supply (Ratcliffe 1997) and was postulated to act as an intracellular messenger to mediate the activation of the H+-ATPase (Reggiani, Zaina and Bertani 1992). Thus, finding QTLs associated with such cell acidosis and regulation of H+-ATPase may be a useful strategy for breeders, especially in the light of the crucial role of H+-ATPase in membrane potential maintenance and control over plant membrane transport activity (Shabala et al. 2014). Another important target maybe hypoxia-induced shifts in cell metabolism and, specifically, interplay between carbohydrate concentrations, alcoholic fermentation, and GABA production (Jaeger et al. 2009, Shabala 2011). GABA content increases dramatically in waterlogged plants (Bailey-Serres and Voesenek 2008). It was shown that differences in GABA conversion into alanine (which might result in an accumulation of phytotoxic levels of intermediates) was a crucial factor differentiating waterlogging stress tolerance among Fraxinus species from different ecophysiological habitats (Jaeger et al. 2009). Also important is the trans-port of sugar to the root system (Drew 1997). All these traits should be considered as potential biochemical tar-gets in breeding programs.

Waterlogging tolerance is not related to GABA and lactic acid contents in roots

GABA is formed with glutamate decarboxylase as a catalyser, with cytosolc H^+ and Ca^{2+} activating the glutamic acid precursor process (Ratcliffe 1997, Shabala et al. 2014). The GABA is considered a mechanism adapting to oxygen deprivation (Drew 1997, Kreuzwieser et al. 2009). The increase in GABA con-tents under waterlogging conditions was reported in both trees (Kreuzwieser, Fürniss and Rennenberg 2002) and Lotus japonicus (Rocha et al. 2010). Similar with the report in trees (Kreuzwieser et al. 2002), we found that GABA could accumulate in both waterlogging-tolerant and waterlogging-sensitive genotypes in barley. Therefore, GABA accumulation under waterlogging stress is un-likely to be a mechanism for differential waterlogging tolerance in barley.

A lack of oxygen in waterlogged roots induces the anaerobic mode of the plants, such as alcoholic and lactic acid fermentation. However, anaerobic respirations were relatively inefficient for energy production and the overproduction of lactic acid can also cause cell death in roots (Drew 1997, Shabala 2011). A correlation between waterlogging tolerance and higher lactic acid fermentation was reported in Limonium (Rivoal and Hanson 1993). In addition, lactic acid efflux also plays an important role for waterlogging tolerance in maize (Xia and Saglio 1992). However, in our study, no associations were found between waterlogging tolerance and lactic acid contents in barley.

In conclusion, waterlogging tolerant genotypes of barley showed not only significantly higher adventitious root porosity than susceptible genotypes but, more importantly, a faster increase of root porosity resulting from faster development of aerenchyma. The percentage of root porosity after 7 days of waterlogging treatment showed the greatest differences among genotypes. We also conclude that antioxidant enzyme activities in leaves of plants with roots under waterlogging conditions cannot be used as selection criteria for waterlogging tolerance.

Chapter 4: Identification of aerenchyma formation-related QTL in barley

Abstract

Waterlogging is one of the important limiting conditions for crop yield and productivity. The main feature of waterlogged soils is oxygen deprivation, due to slow gas diffusion in water. Decreased oxygen content in waterlogged soils leads to the oxygen deficiency in plant tissues, resulting in reduced energy availability for plants. Rapidly induced aerenchyma formation is critical to maintaining adequate oxygen supply and overall waterlogging tolerance in barley. In this study, we have proved that quantifying aerenchyma formation after 7 days of waterlogging in commercial potting mixture can be a reliable, fast, and widely utilised approach for the selection of waterlogging tolerant barley genotypes, which is supported by measurements of redox potential (an indicator of anaerobic conditions). This protocol was also used to identify quantitative trait loci (QTL) in a doubled haploid population of barley from the cross between Yerong (tolerant) and Franklin (sensitive) genotypes. The QTL for aerenchyma formation and root porosity were at the same location as the waterlogging tolerance QTL. Seven new markers were developed and added onto this region on chromosome 4H. One major QTL for aerenchyma formation after 7 days waterlogging treatment explained 42.8% of the phenotypic variance. This successful QTL for aerenchyma formation can be effectively used in the marker assisted selection to improve waterlogging tolerance in barley.

Introduction

Waterlogging is one of the important constraints to crop production. Waterlogging usually occurs in duplex, or texture soils, causing 20% to 25% yield loss of barley (P de San Celedonio et al. 2014, Setter et al. 1999). The annual damage of crops caused by

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waterlogging exceeds 60 billion Euro (www.dartmouth.edu/~floods/Archives/2005sum.htm). In waterlogged soils, gas diffusion is 10,000 fold slower than the gas diffusion in air, greatly decreasing the gas exchange between waterlogged plants and air (Armstrong 1979).The main feature of waterlogged soils is the oxygen deprivation due to slow gas diffusion. Decreased redox potential (Eh) is widely used as the indication of anaerobic conditions in waterlogged soils (Fiedler et al. 2007). Molecular oxygen is the final electron acceptor in the mitochondrial electron transport. Decreased oxygen content in waterlogged soils leads to oxygen deficiency in plant tissues, resulting in reduced energy availability for plants.

During waterlogging plants develop physiological and morphological mechanisms to overcome the energy crisis. One of these alterations is the formation of aerenchyma. Aerenchyma is the gas space formed by cell death or cell wall separation, that improves oxygen transportation from shoots to roots, resulting in higher oxygen contents in waterlogged roots. Some species, such as rice and wild relatives of maize, are able to form constitutive lysigenous aerenchyma under non-waterlogging conditions. The constitutively formed aerenchyma allows cultivars to adapt to waterlogging conditions more rapidly than those without constitutive aerenchyma (Evans 2004). The constitutive root porosity in rice can be 20-30%, increasing to more than 40% in waterlogged soils (Colmer 2003a; Steffens et al. 2010). The formation of aerenchyma in roots allows more oxygen to be stored in root tissue. A higher concentration of oxygen is able to increase the energy production (ATP) in waterlogged plants and avoids the adverse effects by waterlogging (Bailey-Serres and Colmer 2014, Shabala et al. 2014). Root porosity (the percentage of gas volume per root volume) is widely used as the indicator of aerenchyma formation (Colmer 2003b). This is closely correlated with waterlogging tolerance in crops (Colmer and Voesenek 2009). Apart from a high percentage of aerenchyma, faster aerenchyma formation in adventitious roots has also been found in waterlogging tolerant varieties, which is considered one of the key factors for waterlogging tolerance in barley (Zhang et al. 2015b).

Genetic diversity in waterlogging tolerance in barley has been reported (Garthwaite et al. 2003, Setter et al. 1999, Zhou et al. 2007). However, the progress in breeding for waterlogging tolerant commercial varieties is quite slow mainly because of the difficulty in accurate phenotyping (Zhou 2010). Marker assisted selection (MAS) has been proved to be very effective in improving quantitative traits in breeding programs, as molecular markers

give unambiguous, single site genetic differences that can easily be scored and mapped in most segregating populations. Most published QTL studies for waterlogging tolerance were based on morphological and agronomical traits and there is a need to screen waterlogging tolerance with physiological traits (Shabala 2011) so breeders can target specific tolerance mechanisms or pyramid different tolerance genes. For example, higher root porosity and a barrier to radial oxygen loss in *Hordeum marinum* has been successfully introgressed into cultivated wheat to improve waterlogging tolerance (Malik, Islam and Colmer 2011). Wild relatives of maize are able to form constitutive lysigenous aerenchyma under non-waterlogging conditions. One major QTL for aerenchyma formation under aerated conditions was found in different populations on chromosome 1 in maize (Mano and Omori 2008, Mano and Omori 2009, Mano et al. 2008, Mano et al. 2007). Genes have been reported for inducible aerenchyma formation under hypoxia in *Arabidopsis* (Muhlenbock et al. 2007). In barley, a major QTL for root porosity in both aerated and anoxia hydroponic condition was detected on chromosome 4H from the YYXT/Franklin population (Broughton et al. 2015). Major QTL with the LOD value above 3 and the value of phenotypic variance exceeding 10% were selected for meta-analysis, as only QTL with these qualities can potentially be used in marker assisted selection and positional cloning (Collard et al. 2005).The position of this QTL for root porosity was the same as the QTL for waterlogging tolerance from the same population (Zhou et al. 2012).

In this study, we aimed to develop a different approach to score aerenchyma formation (i.e. both root porosity and direct observation of the percentage of aerenchyma formation under waterlogging conditions using potting mixture); to validate the new approach by measuring Eh in waterlogged potting mixture and waterlogged brown sodosol soil which is collected from waterlogged site in Tasmania; and to validate the QTL (Broughton et al. 2015) in a different population using the protocol we developed earlier (Zhang et al 2015). New molecular markers were also developed to fine map this QTL region for further MAS.

Materials and Methods

Redox potential in waterlogged brown sodosol soil and potting mixture

Six barley (*Hordeum vulgare* L.) genotypes (Yerong, Franklin, YSM1, Naso Nijo, Gairdner, and TAM407227) were sown in 50-L bins, filled with either commercial potting mixture or

frequently waterlogged brown sodosol soil from Cressy Research Station, Tasmania, Australia. Waterlogging treatment (keeping the water level just above the soil surface) started at the three-leaf stage. Redox potential (Eh) was measured in each variety, at 0 (the day before waterlogging treatment), 1, 3, 5, 7, and 14 days after waterlogging treatment in both soil types, 5 cm below the soil surface. Each treatment was repeated three times in the glasshouse from February to April, 2014.

Eh was measured with TPS Eh sensor (http://www.tps.com.au). The sensor was platinum tipped with Ag/AgCl as the reference electrode. Before measurements, the accuracy of Eh sensor was tested with Zobells solution (+430 mV), 3.3×10^{-3} M $K_4Fe(CN)_6$ + 3.3×10^{-3} M K_3 $Fe(CN)_6$ in 0.1 M KCl (ZoBell 1946).

Difference in aerenchyma formation among barley genotypes

In addition to the six genotypes (Yerong, Franklin, YSM1, Naso Nijo, Gairdner, and TAM407227) used in above Redox potential experiment, five other genotypes (YYXT, TX9425, YF374, Dayton, and CM72) were also added to this experiment. Seeds were obtained from either the Australian Winter Cereal Collection or China through the joint project "Australia China collaboration on barley germplasm". Among these genotypes, TAM407227 and Yerong are the most waterlogging tolerant genotypes and Franklin and Naso Nijo are the most susceptible genotypes (Zhou 2011, Zhou et al. 2012). Seeds of all genotypes were sown in 50-L bins, filled with a pine bark/loam-based potting mixture with premixed slow release fertiliser, as described by (Zhou 2011), 30 plants per square meter. Waterlogging treatment started at the three-leaf stage. The experiment was repeated three times in the glasshouse in May - June, 2014.

Aerenchyma formation

Seven days after waterlogging treatment, adventitious roots from different genotypes were sampled. Approximately 2 cm long root segments were taken from the mature zone, about 6 cm from the root apex. Cross sections were cut by free-hand with razor blades (Pang et al. 2004) and observed under a bright field light microscope (Olympus BX41). The proportion of aerenchyma was visually scored based on digital images from Olympus DP20: 0 = no

aerenchyma, 4 = well-formed aerenchyma (Fig. 4.1), which is a modified scoring system from (Mano et al. 2006).

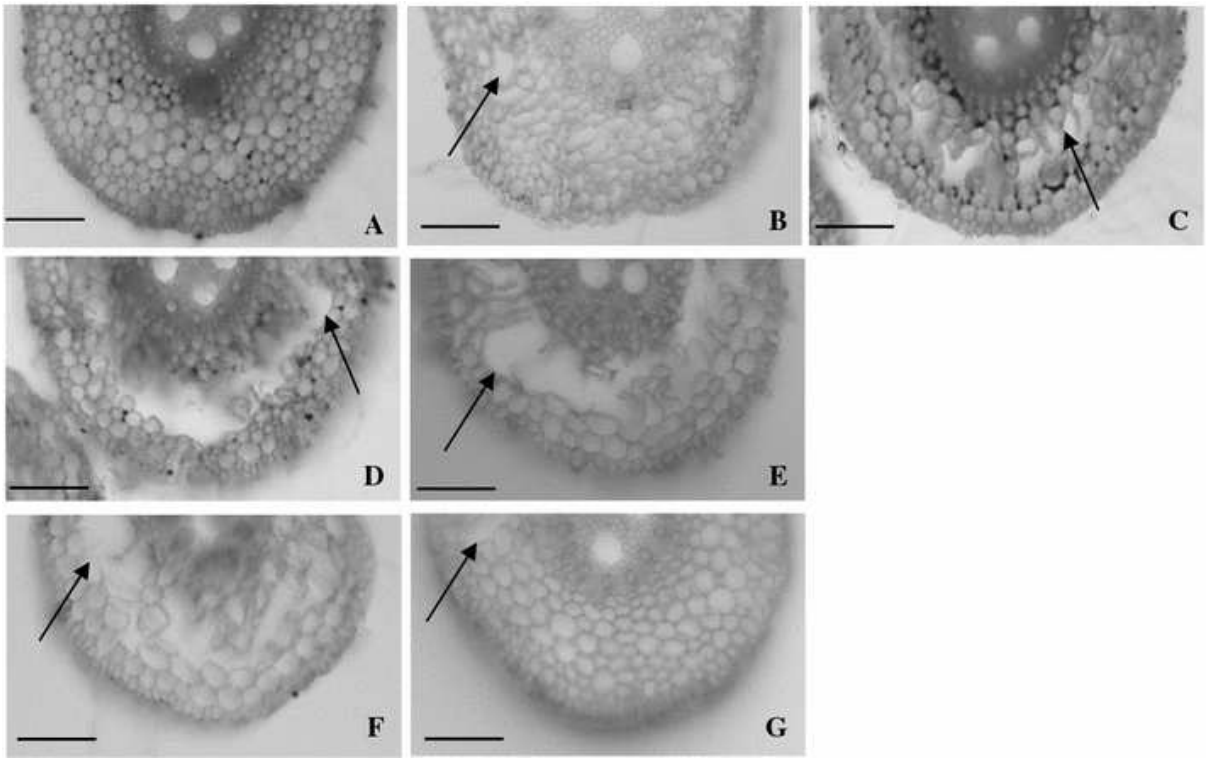

Figure 4.1 Light micrographs of cross section of adventitious roots. After 7 days waterlogging, aerenchyma formation from 0 (no aerenchyma) to 4 (well-formed aerenchyma), respectively from **a** to **e**. Yerong (**f**) had a larger proportion of aerenchyma than Franklin (**g**). *Bar* = 100 µm

Aerenchyma formation and root porosity QTLs under waterlogging conditions

A total of 177 double haploid (DH) lines, from the cross between Yerong and Franklin were used in this study. The parent varieties, Yerong and Franklin, showed significant differences in waterlogging tolerance (Zhou 2011), root porosity and aerenchyma formation under waterlogging stress (Zhang et al. 2015b). DH lines and parent varieties were grown in 50-L bins, filled with pine bark/loam-based potting mixture with premixed slow release fertiliser. Waterlogging treatment began at the three-leaf stage. Aerenchyma formation of all the 177 DH population lines was measured after 7 days waterlogging treatment. The experiment was repeated three times in the glasshouse from July to December, 2014.

Root porosity was also measured by the buoyancy of the adventitious roots before and after vacuum infiltration (Raskin 1983), based on equations modified by Thomson et al. (1990). Seven days after waterlogging treatment, adventitious roots of plants were collected from soils and carefully washed with water. Approximately 0.3 to 0.4 g (fresh weight) of each adventitious roots sample was used for the measurement.

Genetic map construction and QTL analysis

The original molecular map for the Yerong/Franklin population comprised 196 DArT and 28 microsatellite markers (Li et al. 2008). The software package MapQTL 6.0 (Van Ooijen and Kyazma 2009) was used to detect QTL controlling root porosity values and the scores for aerenchyma formation after 7 days of waterlogging. Interval mapping (IM) was used to detect major QTL. The closest marker at each QTL from interval mapping was selected as a cofactor in the multiple QTL model (MQM). Logarithm of the odds (LOD) threshold values of 3.0 was used to detect the presence of a QTL. The linkage maps showing the QTL positions were made with MAPCHART (Voorrips 2002).

The development of new markers

New InDel markers were developed using the method described by (Zhou et al. 2015). The genomic DNA sequences of three barley cultivars Morex, Barke and Bowman (verified on 18 Oct 2012), were downloaded from ftp://ftpmips.helmholtz-muenchen.de/plants/barley/public_data/. Morex contig sequences in the region of our interest were retrieved and used to blast against Barke and Bowman sequence. The DNA sequences from the three cultivars were aligned to explore InDel (insertion and deletion) with the software Genieous. Primers were designed with Genieous in InDel positions. The developed markers were tested for polymorphisms between Yerong and Franklin. Polymorphic markers were used for fine mapping of this QTL.

Results

Redox potential in waterlogged brown sodosol soil and waterlogged potting mixture

There was no significant difference in Eh among different genotypes at different treatment stages in both potting mixture and sodosol soil (Fig. 4.2). Eh of both aerated potting mixture

and aerated brown sodosol soil were above +300 mV, with potting mixture showing slightly but significantly greater Eh than aerated brown sodosol soil ($P < 0.01$). One day after waterlogging, the Eh in the brown sodosol soil decreased to lower than -300 mV while Eh in potting mixture only decreased slightly to about +100 mV. However, 3 days after waterlogging treatment, the Eh of both potting mixture and soil decreased to lower than -300 mV and changed little afterwards, with the Eh of the potting mixture being slightly higher than that of the soil ($P < 0.01$).

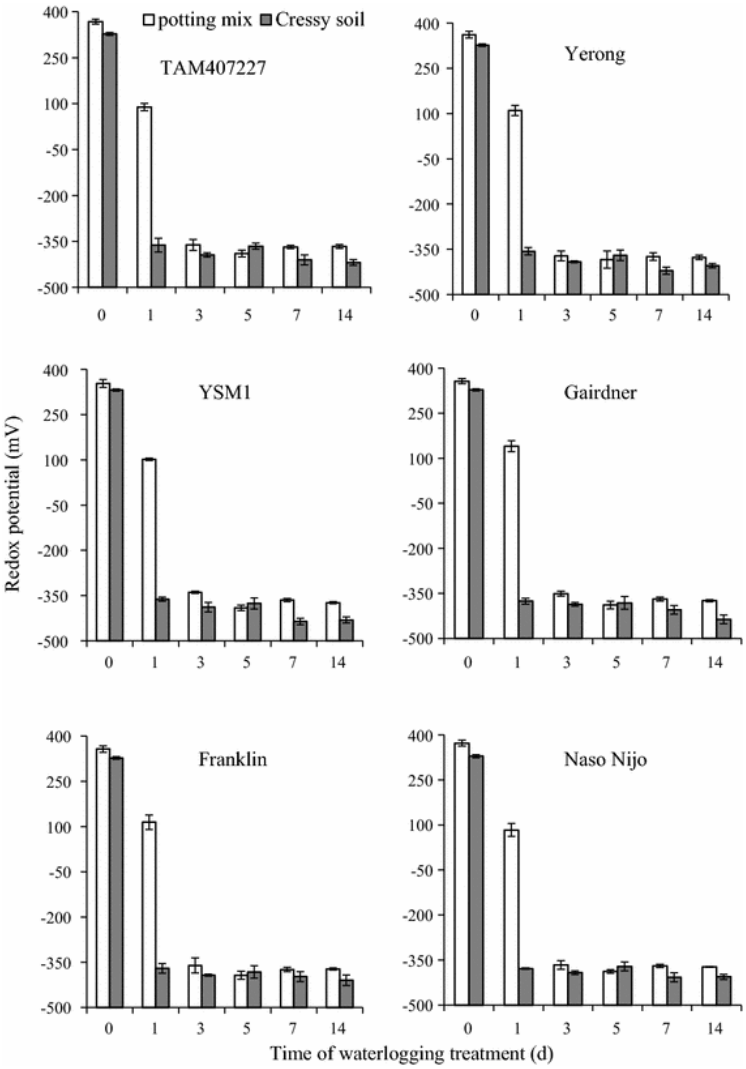

Figure 4.2 Eh of six different barley genotypes after 0, 1, 3, 5, 7, and 14 days of waterlogging in brown sodosol soil and commercial potting mixture. Waterlogging was started at the three-leaf stage of barley. Eh values are the means ± standard deviations of three replicates 5 cm below the soil surface. Each *replicate* represents three parts of soils in different tanks.

Difference in aerenchyma formation among barley genotypes

Eleven barley genotypes showed significant difference in root porosity after 7 days of waterlogging treatment in potting mixture (Table 4.1). The most waterlogging tolerant genotype, TAM407227, had the highest aerenchyma formation with a score of 3.5 (out of a possible maximum value of 4.0) after 7 days of waterlogging. In contrast, the aerenchyma formation of Franklin, which is the most susceptible genotype, was only 0.3 after 7 days waterlogging. Significant and positive correlation ($P < 0.01$) was found between aerenchyma formation, root porosity, and waterlogging tolerance (Zhou 2011, Huang et al. 2014) of these eleven barley genotypes.

Table 4.1 Aerenchyma formation among 11 different barley genotypes after 7 days waterlogging. Waterlogging treatment started at the three-leaf stage of barley plants grown in commercial potting mixture. Values are the mean ± SE of three replicates. Each replicate represents roots from three single plants growing in different tanks.

Genotypes	Aerenchyma formation score	Waterlogging tolerance score
TAM407227	3.5 ± 0.5	9.5 ± 0.5
Yerong	2.8 ± 0.4	8.8 ± 0.5
YSM1	2.3 ± 0.4	6.5 ± 1.0
Gairdner	1.5 ± 0.5	5.8 ± 1.5
Franklin	0.3 ± 0.3	3.0 ± 0.5
Naso Nijo	0.5 ± 0.5	3.8 ± 0.5
YYXT	2.8 ± 0.4	7.0 ± 2.0
TX9425	2.0 ± 0.7	8.0 ± 0.5
YF374	2.0 ± 0.7	8.0 ± 0.5
Dayton	2.0 ± 0.7	5.0 ± 1.0
CM72	3.3 ± 0.7	8.3 ± 1.5

Aerenchyma formation and root porosity QTL under waterlogging conditions

After 7 days of waterlogging, parental line Yerong showed significantly higher aerenchyma formation and root porosity than Franklin (Table 4.1). The 177 DH lines varied significantly in root porosity (Fig. 4.3) and aerenchyma formation (Fig. 4.4) after 7 days of waterlogging

treatment. Both aerenchyma formation and root porosity of the DH lines were significantly correlated with waterlogging tolerance ($P < 0.01$) (Fig.4.5 and Fig. 4.6).

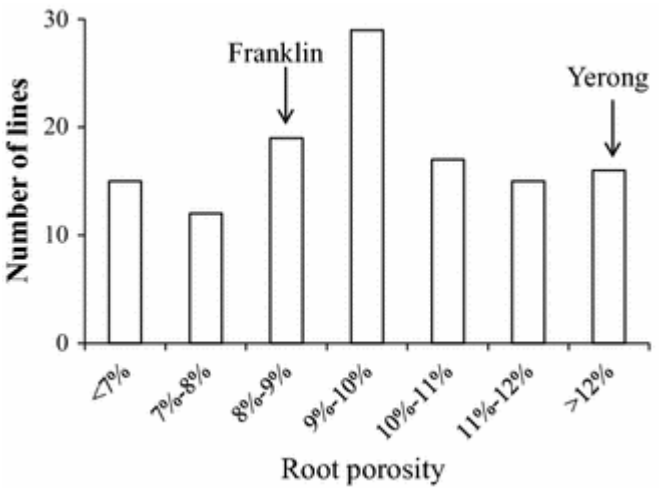

Figure 4.3 Distribution of root porosity of the 177 DH lines derived from the cross of Yerong and Franklin

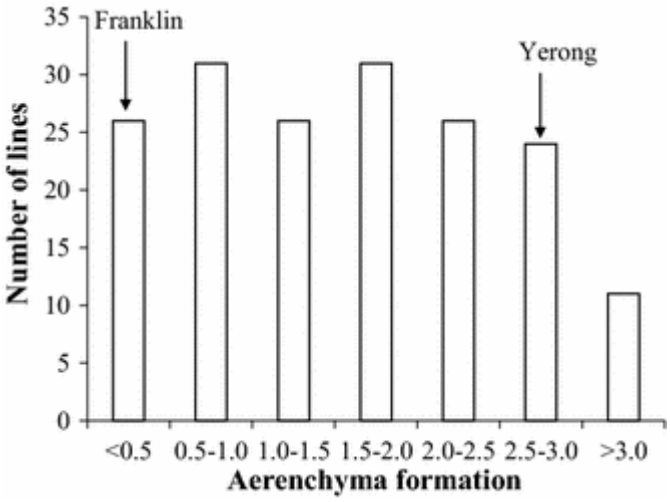

Figure 4.4 Distribution of aerenchyma formation scores of the 177 DH lines derived from this cross as well as aerenchyma formation scores of Yerong and Franklin

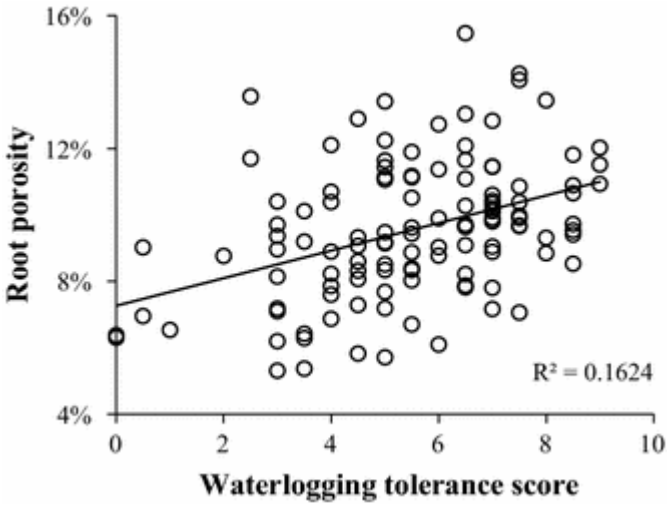

Figure 4.5 Correlation between waterlogging tolerance and root porosity after 7 days of waterlogging among 177 DH lines. Waterlogging tolerance score is based on leaf chlorosis and plant survival after 9 weeks waterlogging treatment (Zhou 2011)

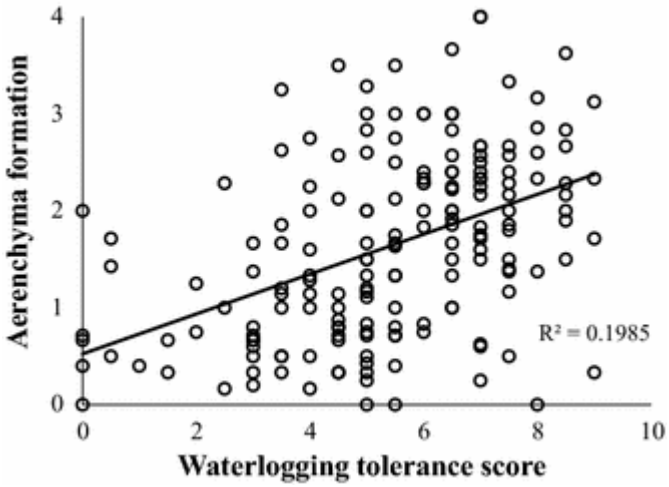

Figure 4.6 Correlation between waterlogging tolerance score and aerenchyma formation score after 7 days of waterlogging among 177 DH lines. Waterlogging tolerance score is based on leaf chlorosis and plant survival after 9 weeks waterlogging treatment (Zhou 2011)

QTL analysis was conducted using the earlier reported genotypic data and map (Li et al. 2008). Two significant QTL were detected for root porosity after 7 days of waterlogging, on chromosome 4H and 6H. A major QTL was identified on chromosome 4H, explaining 21.2% of the phenotypic variation with a LOD value of 6.4. The nearest marker was bPb-8164. Another minor QTL was found on chromosome 6H. Bmag0500 is the nearest marker to the

QTL with a LOD value of 3.7, explaining 10.2% of the phenotypic variation. Only one major QTL was identified for scores of aerenchyma formation. This QTL was located at the same position to that for root porosity. The QTL explained 22.0% of the phenotypic variation with a LOD value of 9.4, with Ebmac0679 being the nearest marker (Table 4.2).

As the major QTL was found at a similar position on 4H from different populations and different scoring methods, candidate genes were searched from this region and some new markers were designed to screen the DH population again. After seven new markers were added to the linkage map of chromosome 4H (Fig.4.7), the percentage of phenotypic variation determined by the QTL increased from 21.2% to 26.2% for root porosity and from 22.0% to 42.8% for aerenchyma score (Fig.4.7) (Table 4.2). GF247 and GF209 are the nearest markers for root porosity and aerenchyma scores. These two markers are much closer to the gene than the previously identified nearest marker, Ebmac0679.

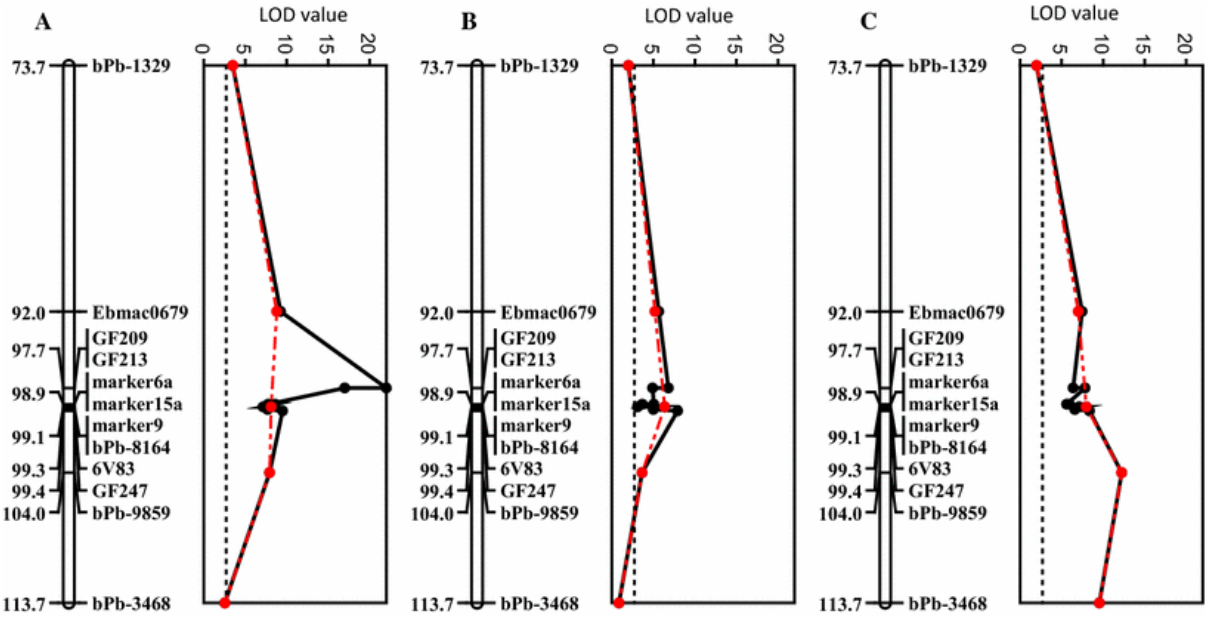

Figure 4.7 QTL for aerenchyma formation (**a**) and root porosity (**b**) after 7 days waterlogging and QTL for waterlogging tolerance (**c**) on chromosome 4H. *Red dashed line* results before fine mapping. *Black solid line* results after fine mapping. The positions of all the selected markers were adjusted to those in physical map (http://phymap.ucdavis.edu:8080/barley/)

Table 4.2 Fine mapping of aerenchyma formation, root porosity under waterlogging conditions and waterlogging tolerance

Position	Marker	LOD	Fine mapped LOD	R^2 (%)	Fine mapped R^2 (%)
71.3	HVM68	2.6	2.8	6.7	7.0
73.4	bPb-3717	1.1	1.6	2.9	4.1
78.6	bPb-3468	2.9	2.5	7.4	6.4
78.8	bPb-1329	3.7	3.2	9.2	8.1
92.2	GF209		21.2		42.8
96.5	bPb-9859	7.8	8.0	18.6	18.9
101.2	Ebmac0679	9.4	9.4	22.0	22.0
103.2	bPb-8164	8.1	8.1	19.3	19.3
106.2	6V83		7.5		17.8
107.9	GF247		9.1		21.3
111.1	marker6a		8.0		19.0
114.5	marker15a		9.1		21.4
117.2	marker9		7.6		18.2
123.1	GF213		15.7		33.8

To further confirm the relationship between aerenchyma formation/root porosity and waterlogging tolerance, specifically in this QTL region, QTL for waterlogging tolerance was re-analysed using either aerenchyma formation or root porosity as a covariate. When root porosity was used as a covariate, the percentage of phenotypic variation (R^2) explained by the QTL on 4H for waterlogging tolerance decreased from 27.4% to 17.5%. Similarly, when aerenchyma score was used as a covariate, the R^2 explained by the QTL on 4H for waterlogging tolerance decreased from 27.4% to 13.4%. The results suggest a close relationship between aerenchyma formation and waterlogging tolerance.

Discussion

Protocol for early detection of waterlogging tolerance

Eh is considered an effective measure to test the oxygen conditions in waterlogged soils (Fiedler et al. 2007). Significant decreases in Eh under waterlogging were found in different soils including potting mixture (Mano and Takeda 2012), crowley silt loam (Reddy and Patrick jr 1976) and other soils (Unger, Motavalli and Muzika 2009). In the current research, the brown sodosol soil was from Cressy research station in Tasmania, Australia, where waterlogging occurs frequently. Waterlogging stress caused severe anaerobic conditions in

brown sodosol soil, reflected by the decrease of Eh (from around +300 mV to less than -300 mV). Similarly waterlogging also caused the decrease of Eh in potting mixture but the rate of decreasing was much slower than that in brown sodosol soil in the first two days. However, three days after waterlogging, the Eh in both brown sodosol soil and potting mixture decreased to under -300 mV and remained steady for the rest of the experiment.

Accurate phenotyping is the major constraint in identifying the QTL for waterlogging tolerance due to the complexity of waterlogging tolerance mechanisms and different waterlogging environmental conditions (Zhou 2010). The evaluation of waterlogging tolerance based on leaf chlorosis and survival rate has been demonstrated to be a highly reliable screening method (Zhou 2011). Waterlogged commercial potting mixture with 0.1% soluble starch was another reliable and fast method to select waterlogging tolerant barley genotypes based on leaf injury after 14 days waterlogging treatment (Mano and Takeda 2012). Our earlier experiments have shown that aerenchyma formation based on root porosity in potting mixture under waterlogging stress was significantly correlated with waterlogging tolerance (Zhang et al. 2015b). However, the measurement of root porosity is time consuming and labour intensive. In this experiment, we have developed a faster and highly reproducible protocol to identify aerenchyma formation by direct scoring of aerenchyma. This method was compared with root porosity measurement. In general, these two traits were closely correlated ($R^2 = 0.27$). However, the errors for measuring root porosity were much greater than those for direct scoring aerenchyma. Aerenchyma scores not only showed better correlation with waterlogging tolerance, but the phenotypic variance determined by the QTL for aerenchyma formation was also much higher than that for root porosity. In addition, the direct scoring of aerenchyma is much faster (up to 60 samples a day, compared with only 20 samples a day for root porosity). Thus, direct scoring of aerenchyma is a better protocol for routine screening.

Aerenchyma formation and root porosity QTL are related to waterlogging tolerance QTL in barley

Marker assisted selection (MAS) is an effective approach to select stress tolerant genotypes without environment effects. Many QTL have been successfully used in breeding programs for introgressing and pyramiding major effect genes (Septiningsih et al. 2009, Xu and Crouch 2008). However, little progress has been achieved to complex traits such as waterlogging, drought tolerance, and salinity tolerance. Efforts have been made to dissect complex traits

into relatively simpler traits that are controlled by one or two genes (Shabala 2011). Plant waterlogging tolerance is also influenced by many mechanisms including aerenchyma formation (Evans 2004), developing adventitious roots (Garthwaite et al. 2003), and low radial oxygen loss (Colmer and Voesenek 2009). Among them, aerenchyma formation under waterlogging stress is one of the major mechanisms. In this study, all the DH lines with high aerenchyma formation showed at least medium tolerance to waterlogging (Fig 4.6). Some DH lines with low aerenchyma formation also showed the tolerance to waterlogging, indicating that other mechanisms also contribute to the tolerance.

A major QTL for root porosity under waterlogging conditions was identified from a DH population between the cross of YYXT (waterlogging tolerant) and Franklin (Broughton et al. 2015). This QTL was located at the same position as a QTL for waterlogging tolerance on chromosome 4H (Zhou et al. 2012). However, the QTL for waterlogging tolerance only explained around 5% of the phenotypic variation. In this experiment, a different DH population was used to validate the QTL with the newly developed screening method. The major QTL for both direct scores of aerenchyma formation and root porosity was located on 4H at a similar position as the one identified from the YYXT/Franklin population (Broughton et al. 2015). A minor QTL for root porosity on chromosome 6H was located at a similar position to the one for waterlogging tolerance at early growth/waterlogging treatment stages (Zhou 2011). Further QTL analysis for waterlogging tolerance using root porosity/aerenchyma score as covariates confirmed the contribution of aerenchyma formation to overall waterlogging tolerance.

The correlation between waterlogging tolerance scored in the field (Zhou et al 2010) and increased root porosity in the YYXT/Franklin population was weak ($P < 0.1$) (Broughton et al. 2015). There was a significant correlation ($P < 0.01$) between waterlogging tolerance from the same field trial and increased aerenchyma formation and root porosity in the Yerong/Franklin population. However, these protocols are different. We measured aerenchyma formation and root porosity after 7 days of waterlogging in commercial potting mixture from the Yerong/Franklin population, while root porosity was measured after 20 days anoxia treatment from the YYXT/Franklin population (Broughton et al. 2015). It is probable that waterlogging tolerance is better correlated with faster formation of aerenchyma

under waterlogging stress, compared with the root porosity of plants after more than 20 days waterlogging.

Fine mapping of the QTL for aerenchyma formation on chromosome 4H

Fine mapping is widely used to refine the QTL positions for successful MAS and searching for candidate genes (de Dorlodot et al. 2007, Semagn et al. 2013). In our study, seven polymorphic InDel markers were developed to fine map the major QTL for aerenchyma formation under waterlogging conditions. By genotyping the DH population, 27 recombinant lines were identified in the QTL region. These recombinant lines were evaluated for their root porosity again. By comparing the genotypes and phenotypes of these lines (77, 92, 107, 158, and 213), it can be concluded that the gene controlling aerenchyma formation should be located on the right side of the marker GF199. Based on the genotypes and phenotypes of lines 287, 322 and 344, the gene should be on the left side of the marker GF211. Thus the QTL can be fine-mapped to the region between GF199 (80.95 cM) and GF211 (99.08 cM) (Table 4.3).

Table 4.3 Genotyping of recombinant DH lines from the cross of Franklin and Yerong (color table online)
Green blocks indicate that the fragments are from Yerong, while Red blocks indicate that the fragments are from Franklin. R represents resistant; S represents susceptible; M represents medium tolerance. Morex contig sequences in the region of our interest were retrieved and used to blast against Barke and Bowman sequence. The DNA sequences from the three cultivars were aligned to explore InDel (insertion and deletion) with the software Genieous. Primers were designed with Genieous in InDel positions. The developed markers were tested for polymorphisms between Yerong and Franklin and were used for fine mapping of this QTL.

Lines	GF199	GF207	GF209	GF317	GF213	GF211	GF299	GF243	GF247	Phenotype	R/S/M
Pos.(cM)	80.95	91.71	97.66	97.6	97.66	99.08	99.08	99.33	99.43		
Yerong										2.8	R
Franklin										0.3	S
Line 22										1.8 ± 1.2	R
Line 52										1.8 ± 0.6	R
Line 119										1.6 ± 0.4	R
Line 147										1.4 ± 0.8	R
Line 151										2.1 ± 1.2	R
Line 160										2.1 ± 1.0	R
Line 190										2.6 ± 1.2	R
Line 215										2.4 ± 0.4	R
Line 219										3.6 ± 0.4	R
Line 234										1.7 ± 1.0	R
Line 245										1.6 ± 0.4	R
Line 258										1.6 ± 1.2	R
Line 170										1.5 ± 1.0	M
Line 167										3.5 ± 0.8	R
Line 287										3.3 ± 0.4	R
Line 322										2.8 ± 1.0	R
Line 344										2.1 ± 1.0	R
Line 256										1.1 ± 1.1	M
Line 148										1.5 ± 1.1	M
Line 77										0.6 ± 0.4	S
Line 212										1.0 ± 1.0	S
Line 92										0.7 ± 0.6	S
Line 107										0.5 ± 0.5	S
Line 158										0.6 ± 0.4	S
Line 213										0.3 ± 0.4	S
Line 342										1.1 ± 0.6	S
Line 347										0.8 ± 0.7	S

Barley genomic sequences (ftp://ftpmips.helmholtz-muenchen.de/plants/barley/public_data/) have greatly facilitated the development of new molecular markers. In our future research, more markers will be developed in the region between the markers GF199 and GF207 to further fine map the QTL. Gene annotations (ftp://ftp.ipk-gatersleben.de) will also facilitate fine mapping of this gene. F2 or BCF2 populations will be developed to identify more recombinant lines.

In conclusion, we developed a reliable protocol for early detection of aerenchyma formation under waterlogging conditions. One major QTL for aerenchyma formation and root porosity after 7 days waterlogging was validated in a different population with the gene for fast aerenchyma formation gene originating from a different parent. This major QTL was fine mapped with new developed molecular markers. These new markers can be more effectively used for MAS in barley breeding.

Chapter 5: A new major allele for waterlogging tolerance in wild barley

Abstract

Waterlogging is one of the major abiotic stresses that dramatically reduces barley crop yield. Direct selection on waterlogging tolerance in the field is less effective due to its viability to environment. The most effective way of selection is to choose traits that make significant contributions to the overall tolerance and are easy to score simultaneously. Aerenchyma formation under waterlogging stress is one of the most effective mechanisms to provide adequate oxygen supply and overcome stress-induced hypoxia imposed on plants. In this study, a new allele for aerenchyma formation was identified from a wild barley accession TAM407227 on chromosome 4H. Compared to that identified in cultivated barley, this allele not only produced a greater proportion of aerenchyma but made a greater contribution to the overall waterlogging tolerance. The QTL explained 76.8% of phenotypic variance in aerenchyma formation with a LOD value of 51.4. Markers co-segregating with the trait were identified and can be effectively used in marker assisted selection.

Introduction

Waterlogging dramatically reduces the yield of crops and the problem is exaggerated by the need to increase crop production to feed increasing human population. The yield of barley is dramatically reduced under waterlogging stress (P de San Celedonio et al. 2014) and the cost caused by waterlogging is more than 60 billion Euro annually (www.dartmouth.edu/~floods/Archives/2005sum.htm).

Waterlogging mainly results from heavy rainfall and poor soil drainage (Voesenek, van Veen and Sasidharan 2014, Zhang et al. 2015a). Gas diffusion under waterlogging stress is ten thousand-fold slower than that in air (Armstrong 1979), resulting in a lack of oxygen in waterlogged plants (Voesenek et al. 2016). Aerenchyma formation is one of the mechanisms

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to overcome waterlogging stress. Aerenchyma is the gas space in cortical tissues that improves oxygen transportation from shoots to waterlogged roots (Colmer 2003b). In many wetland species, aerenchyma is well developed even in drained conditions, and can be further enhanced under waterlogging stress (Evans 2004). In barley, waterlogging tolerant genotypes are able to form inducible lysigenous aerenchyma under waterlogged conditions (Zhang et al. 2015b). Aerenchyma formation is also the most effective mechanism for waterlogging tolerance in barley.

The development of waterlogging tolerant varieties is an effective and economical approach to improve crop production under waterlogging conditions. However, the progress of developing waterlogging tolerant barley varieties is slow due to the complexity of waterlogging conditions resulting from different water depth, soil type, duration of waterlogging, nutrient ions and temperature (Setter and Waters 2003, Setter et al. 2009, Zhang et al. 2015b). Waterlogging tolerance is also a complex trait, controlled by many genes including some with small effects (Zhou 2010). Molecular markers have provided plant breeders with a method to improve selection accuracy and accelerate breeding programs.

Many QTL for waterlogging tolerance in barley have been detected (Li et al. 2008, Zhang et al. 2016c, Zhou et al. 2012); however, accurate phenotyping remains the main challenge for improving waterlogging tolerance in breeding. Different traits were used in different studies, such as leaf scoring system, aerenchyma formation and other agronomic traits (Zhang et al. 2016b). The leaf scoring system and aerenchyma formation under waterlogging conditions have been shown to be the most reliable traits for screening waterlogging tolerance in barley (Zhang et al. 2016c, Zhou 2011).

Wild relatives of cultivated crop species are often used as the donor parents in breeding because of their tolerance to biotic and abiotic stresses. Wild relatives of maize are able to form constitutive aerenchyma under aerated conditions (Mano and Omori 2013). This favourable trait for waterlogging tolerance has been successfully used to improve waterlogging tolerance of maize (Mano and Omori 2013, Mano and Omori 2015). Higher root porosity and lower radial oxygen loss from a wild relative *Hordeum marinum* was successfully transferred into cultivated wheat to improve waterlogging tolerance (Malik et al. 2011). The wild barley TAM407227 showed significantly higher potential for enhancing waterlogging tolerance in barley (Zhang et al. 2015b). Compared with waterlogging tolerant

cultivated barley, TAM407227 performed much better with regard to the tolerance to waterlogging with a greater proportion of aerenchyma formation under waterlogging conditions.

In this study, a new linkage map between cultivated barley Franklin and wild barley TAM407227 was constructed. A number of different QTL for different traits under waterlogging and control conditions were detected from this population. Importantly, a new major allele showed much greater effect on aerenchyma formation and waterlogging tolerance and is an ideal candidate gene for use in barley breeding programs.

Materials and Methods

Evaluation of waterlogging tolerant traits

A total of 163 double haploid (DH) lines, from the cross between Franklin and wild barley TAM407227 were used in this study. The wild barley TAM407227 showed better waterlogging tolerance and aerenchyma formation than the cultivated tolerant barley Yerong (Fig. 5.1) (Zhang et al. 2015b). Franklin is a malting barley but susceptible to waterlogging stress. DH lines and parent varieties were grown in a well-constructed field screening facility. Waterlogging treatment began at the three-leaf stage. Waterlogging tolerance was scored based on plant survival and leaf senescence (Zhou 2011). At maturity, different traits were measured for each DH line and parent variety under both control and waterlogging conditions. The traits include plant height under control (CPH) and waterlogging (WPH), the number of tillers under control (CT) and waterlogging (WT), and grain yield under control (CY) and waterlogging (WY) conditions. Relative changes (waterlogging/control) in differences are also used as waterlogging tolerance indicators.

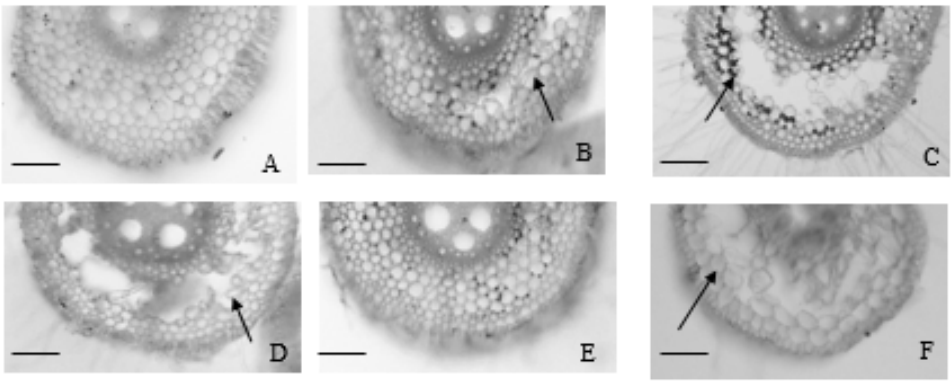

Figure 5.1 Light micrographs of cross section of adventitious roots demonstrating aerenchyma formation (arrows) after 7 days waterlogging treatment in different DH lines: TAMF42 (A), TAMF56 (B), and TAMF135 (C). Wild barley TAM407227 (D) had larger proportion of aerenchyma than Franklin (E) and Yerong (F). Bar = 100 µm.

Aerenchyma formation

Aerenchyma formation of DH lines and parent varieties were detected based on the method by (Zhang et al. 2016c). Plants were grown in 50-L bins, filled with pine bark/loam-based potting mixture with premixed slow release fertiliser. At the three leaf stage, adventitious roots were sampled in each DH line after seven days of waterlogging. Approximately 2 cm long root segments were taken from the mature zone and about 6 cm from the root apex. Cross sections were cut by free-hand using razor blades and examined under a bright field light microscope (Olympus BX41). The proportion of aerenchyma was visually scored based on digital images from Olympus DP20: 0 = no aerenchyma, 4 = well-formed aerenchyma (Zhang et al. 2016c).

Genetic map construction

Diversity Arrays Technology (DArT) assays and SNP markers were developed and conducted by Triticarte Pty. Ltd. A total of around 15,000 DArT markers and 14,500 SNP markers were shown to be polymorphic between Franklin and TAM407227. JoinMap 4.0 was used in this study to construct the linkage map (Van Ooijen and Kyazma 2009). Before map construction, markers with more than 10% missing data and duplicate markers (markers

located at same/similar positions) were deleted. The relatively lower resolution map was used to conduct the preliminary QTL analysis. Further high resolution mapping was conducted in the region on 4H where the major QTL was located.

QTL analysis

The software package MapQTL 6.0 was used to identify different QTL (Van Ooijen and Kyazma 2009). After interval mapping (IM), the closest marker at each QTL was selected as a cofactor in the multiple QTL model (MQM). Logarithm of the odds (LOD) threshold values of 3.0 was used to detect the presence of a QTL. To determine the effects of waterlogging tolerance on other traits, different QTL were re-analysed by using various traits as covariates. The percentage of variance explained by each QTL (R^2) was obtained with restricted MQM mapping. The linkage maps showing the QTL positions were made with MAPCHART (Voorrips 2002). The sequence of flanking SNP markers were used to check the position of QTL on the barley physical map (http://barleygenomeapplications.com/default_2.aspx).

Results

Waterlogging tolerance related traits of DH lines

Franklin and TAM407227 showed significant difference ($P < 0.01$) in plant height, tiller number and grain yield under both waterlogging and control conditions (Table 5.1) with TAM407227 showing significantly higher aerenchyma formation as well as waterlogging tolerance based on plant survival ($P < 0.01$) (Fig. 5.1). Compared with Franklin, TAM407227 had significantly higher plant height, more tillers, but lower yield under control conditions ($P < 0.01$). However, in terms of relative change of traits, TAM407227 was less affected by waterlogging stress. In DH populations, waterlogging stress reduced plant height, tiller number and yield significantly ($P < 0.01$). DH lines showed a wide segregation in waterlogging tolerance. Figure 5.2 shows frequency distributions of waterlogging tolerance based on plant survival and aerenchyma formation in all DH lines. As shown in Table 5.2, waterlogging tolerance showed very high positive correlations with both grain yield ($r = 0.70$, $P<0.01$) and aerenchyma formation ($r = 0.63$, $P<0.01$) under waterlogging stress. Waterlogging tolerance was also significantly correlated with both plant height and tiller

number under waterlogging stress as well as with relative plant height, relative tiller numbers and relative grain yield (Table 5.2).

Table 5.1 Phenotypic values of traits measured in the DH population, Franklin and TAM407227

	Aerenchyma	Waterlogging tolerance	CPH (cm)	WPH (cm)	WCPH	CY (g)	WY (g)	WCY	CT	WT	WCT
Average	1.7	4.3	101.0	69.4	0.7	74.7	34.3	0.5	6.9	3.1	0.5
Min	0.0	0.0	70.0	44.0	0.1	20.9	10.5	0.1	5.0	1.0	0.2
Max	4.0	7.6	131.3	102.5	0.6	126.8	82.2	0.9	9.0	4.8	0.8
Franklin	0.3	0.0	95.0	37.5	0.4	80.4	21.2	0.3	5.0	2.0	0.4
TAM407227	3.5	6.0	121.7	115.0	0.9	58.2	38.0	0.7	7.0	3.3	0.5

The traits include plant height under control (CPH) and waterlogging (WPH), the number of tillers under control (CT) and waterlogging (WT), and grain yield under control (CY) and waterlogging (WY) conditions. Relative changes (waterlogging/control) in differences are also used as waterlogging tolerance indicators.

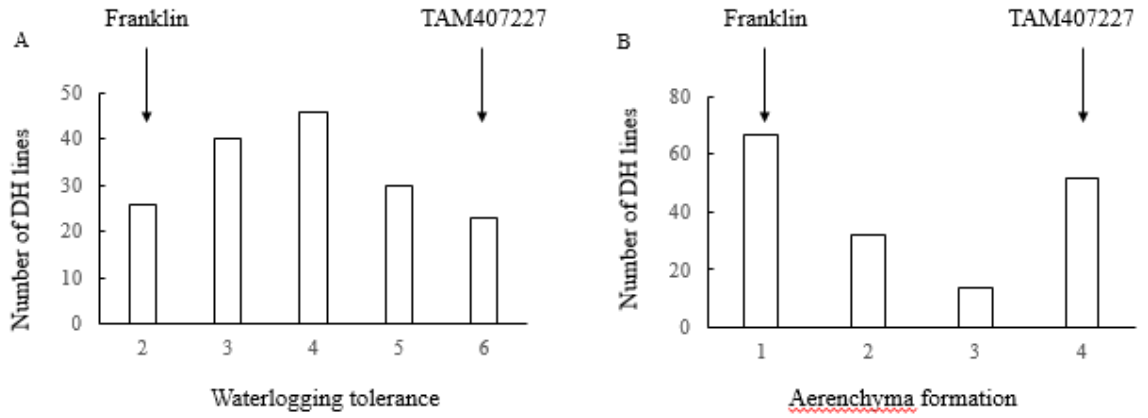

Figure 5.2 Frequency distribution for waterlogging tolerance (A) and aerenchyma formation (B) in DH lines derived from the cross of Franklin and TAM407227.

Table 5.2 Correlation between waterlogging tolerance and all other traits measured in DH population. *, $P < 0.01$ The traits include plant height under control (CPH) and waterlogging (WPH), the number of tillers under control (CT) and waterlogging (WT), and grain yield under control (CY) and waterlogging (WY) conditions. Relative changes (waterlogging/control) in differences are also used as waterlogging tolerance indicators.

	Waterlogging tolerance
Aerenchyma formation	0.63*
WPH	0.26*
WCPH	0.52*
WY	0.70*
WCY	0.54*
WT	0.47*
WCT	0.39*

Figure 5.3 shows the correlation between waterlogging tolerance and aerenchyma formation under waterlogging stress. Aerenchyma formation was clearly grouped into two clusters, one with the scores of more than 2 and the other with the scores of less than 2. In general, nearly all the lines with high scores of aerenchyma formation showed good waterlogging tolerance. However, a few lines with low scores of aerenchyma also showed good waterlogging tolerance, indicating the possible existence of some other tolerance mechanisms.

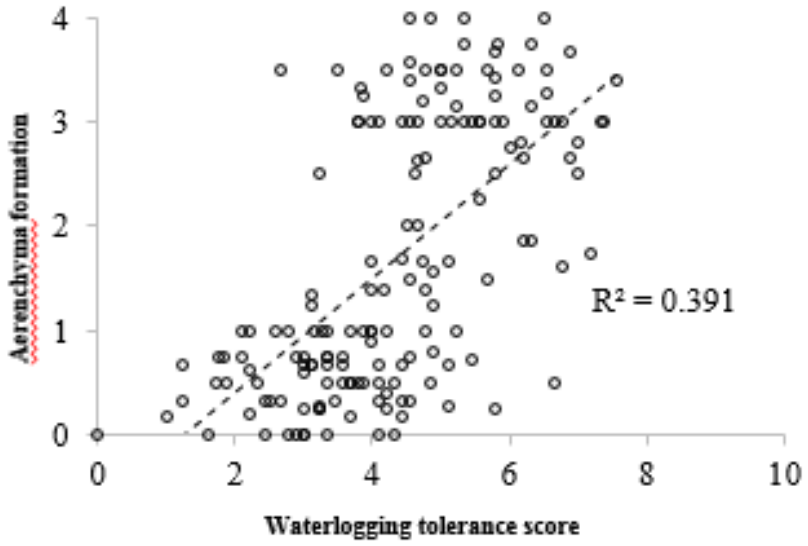

Figure 5.3 Correlation between waterlogging tolerance score and aerenchyma formation after 7 days of waterlogging treatment among 165 DH lines.

QTL for waterlogging tolerance

A total of 19 QTL were identified for different traits under control and waterlogging conditions (Table 5.3 and Fig. 5.4). Three QTL for waterlogging tolerance were based on plant survival. One major QTL was on chromosome 4H at 98.8 cM, with a LOD value of 19.2, explaining 34.6% of the phenotypic variance. Another two minor QTL for waterlogging tolerance were found on chromosomes 6H and 7H, determining 6.3% and 5.3% of the phenotypic variance, respectively. Only one major QTL for aerenchyma formation under waterlogging conditions was identified on chromosome 4H, explaining 76.8% of the phenotypic variation with a LOD value of 51.4. This QTL was located at the same position of a QTL for waterlogging tolerance. A high resolution map on chromosome 4H was further constructed (Fig. 5.5) and the QTL was mapped to the region between 97.5 cm to 99.10 cm on the published consensus map with around 20 markers co-segregating with the traits.

Table 5.3 QTL for waterlogging tolerance related traits under different conditions

Traits	QTL	Chromosome	LOD score	Phenotypic variance	Physical map position	2-LOD interval	Nearest marker
Aerenchyma formation	QTL-AER	4H	51.4	76.8	98.8	99.2-99.3	3255355S4
Waterlogging tolerance	QTL-WL-4H	4H	19.2	34.6	98.8	96.1-104.2	3254813S4
	QTL-WL-6H	6H	4.4	6.3	24.5	16.6-32.8	3260661S6
	QTL-WL-7H	7H	3.7	5.3	70.7	55.5-74.5	3256877S7
Control plant height	QTL-CPH-3H	3H	32.9	60.8	108.4	103.0-105.7	3255136S3
Waterlogging plant height	QTL-WPH-3H	3H	13.0	31.0	117.6	102.1-113.2	4011783S3
	QTL-WPH-4H	4H	3.9	7.2	103.7	102.2-119.5	6437034D
Waterlogging/Control plant height	QTL-WCPH-3H	3H	3.6	7.6	83.6	63.9-109.3	3274569D
	QTL-WCPH-4H	4H	9.4	21.8	98.8	94.4-96.1	7934461D4
Control yield	QTL-CY-3H	3H	9.8	20.3	109.8	98.4-110.6	3254867S3
	QTL-CY-5H	5H	3.0	5.1	114.9	78.4-110.1	3255097S5
	QTL-CY-7H	7H	5.7	11.1	48.7	31.1-65.2	6277000S7
Waterlogging yield	QTL-WY-3H	3H	3.1	7.0	105.9	86.9-110.6	3264662S3
	QTL-WY-4H	4H	9.2	25.0	98.8	96.1-102.4	3255355S4
Waterlogging/Control Yield	QTL-WCY-4H	4H	5.2	12.7	98.8	86.9-100.3	3254813S4
	QTL-WCY-7H	7H	4.0	9.7	126.3	116.1-124.1	3258238S7
Waterlogging tillers	QTL-WT-4H	4H	4.2	11.3	100.0	102.0-104.2	7244354D4
Waterlogging/Control tillers	QTL-WT-4H	4H	5.0	12.7	98.8	84.3-104.3	3254813S4
	QTL-WCT-5H	5H	4.1	10.3	98.1	73.6-92.0	3257423S5

Six other QTL for WPH, WCPH, WY, WCY, WT and WCT were also identified on chromosome 4H at the same position as those for waterlogging tolerance and aerenchyma formation. Another cluster of QTL was found on chromosome 3H at 110 cM based on the barley physical map, controlling CPH, WPH, WCPH, CY and WY (Fig. 5.4).

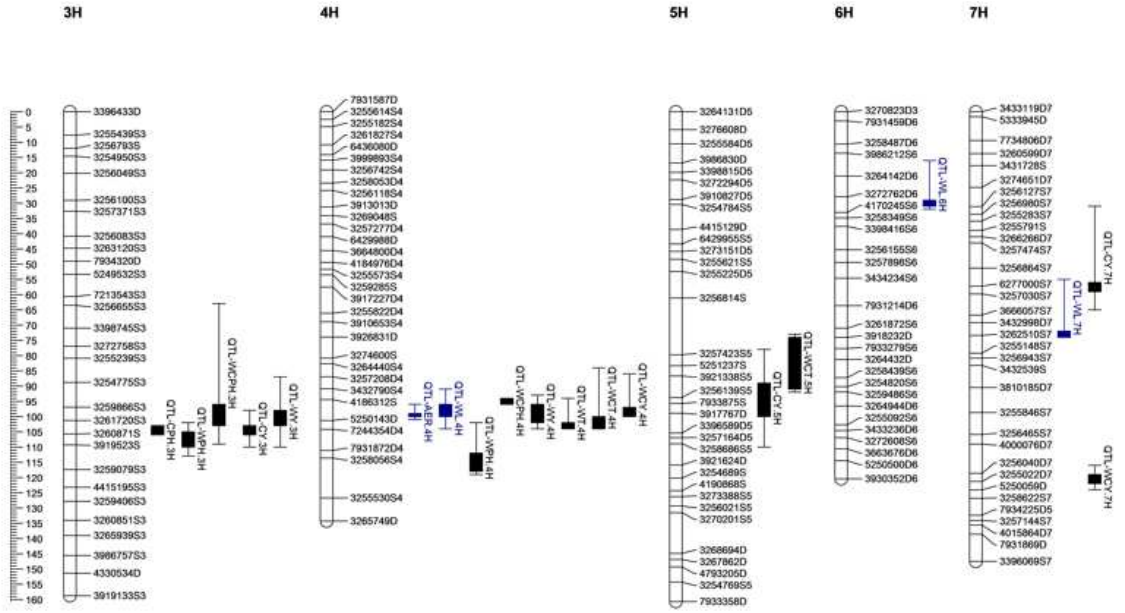

Figure 5.4 Genetic linkage map of Franklin/TAM407227 and QTL identified for different traits in the population. Only selected markers are shown.

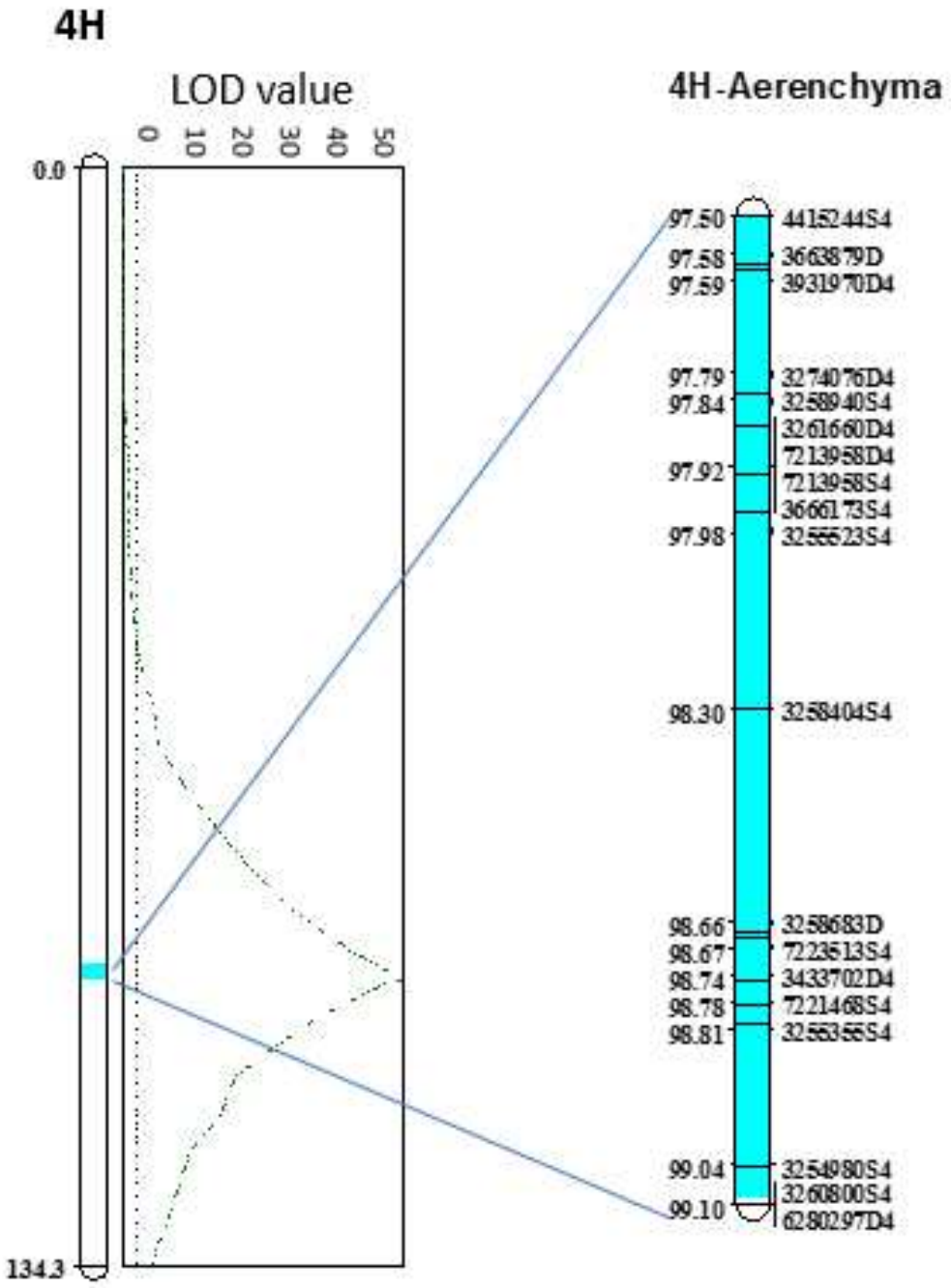

Figure **5.5** QTL for aerenchyma formation after 7 days of waterlogging treatment on a high resolution map of chromosome 4H. All the markers are projected on the barley physical map position.

The contribution of aerenchyma formation to overall waterlogging tolerance

Figure 5.4 shows that the QTL for aerenchyma formation under waterlogging stress is located on a similar position to the major QTL for waterlogging tolerance, WPH, WCPH, WT, WCT, WY and WCY. To confirm their relationships between aerenchyma formation and other

waterlogging tolerance related traits, the scores for aerenchyma formation was used as a covariate while analysing QTL for other traits. Aerenchyma formation under waterlogging made a significant contribution to waterlogging tolerance, as the major QTL on 4H for traits that are used as the indices for waterlogging tolerance became insignificant (Table 5.4 and Fig. 5.6). In contrast, the QTL for these traits on other chromosomes were not affected by using aerenchyma formation as a covariate. Waterlogging scores based on plant healthiness also showed to be a good indicator of grain yield under waterlogging condition. When using the tolerance scores as covariates, QTL for grain yield, plant height and tiller numbers under waterlogging on 4H all became insignificant (Table 5.4). Similarly, waterlogging scores contributed less to QTL on other chromosomes for these traits.

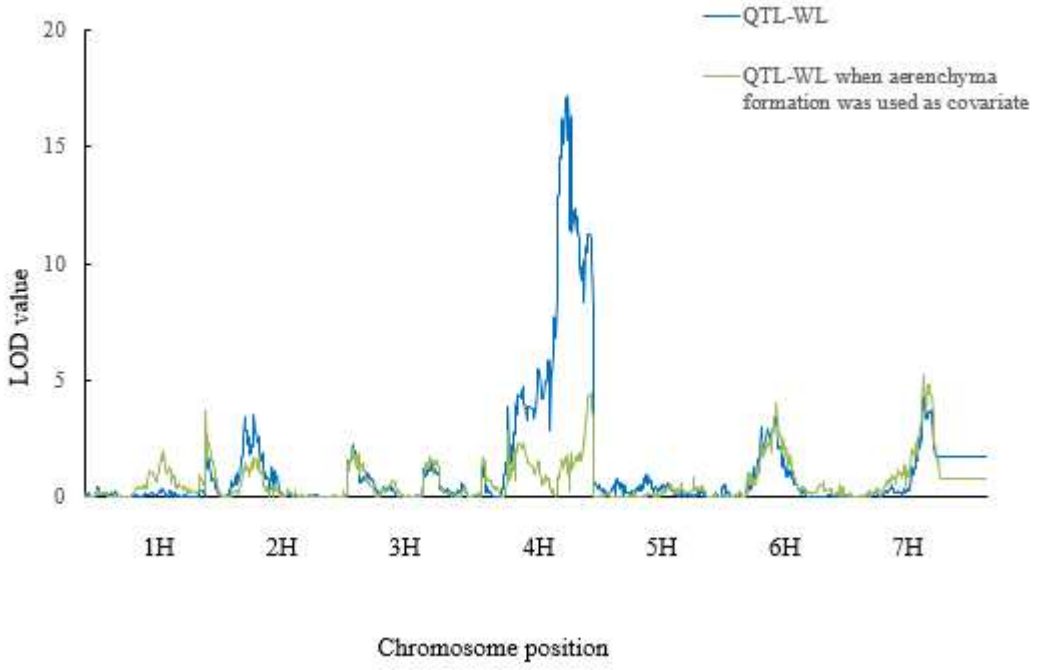

Figure 5.6 QTL for waterlogging tolerance (blue line) and QTL for waterlogging tolerance when aerenchyma formation is used as a covariate (green line).

Table 5.4 Changes in the significance and percentage variation determined by the QTL after aerenchyma or waterlogging tolerance scores were used as covariates. ns: not significant

QTL	Covariate	Linkage group	Nearest marker	Position	LOD	R^2
QTL-WL-4H	Aerenchyma formation				ns	ns
QTL-WL-7H	Aerenchyma formation	7H	3434116S7	63.9	5.3	8.5
QTL-WL-6H	Aerenchyma formation	6H	3260661S6	31.1	4.1	6.7
QTL-WPH-3H	Aerenchyma formation	3H	3917582D	111.8	14.4	31.7
QTL-WPH-4H	Aerenchyma formation				ns	ns
QTL-WCPH-3H	Aerenchyma formation				ns	ns
QTL-WCPH-4H	Aerenchyma formation				ns	ns
QTL-WY-3H	Aerenchyma formation				ns	ns
QTL-WY-4H	Aerenchyma formation				ns	ns
QTL-WCY-4H	Aerenchyma formation				ns	ns
QTL-WCY-7H	Aerenchyma formation	7H	3258238S7	121.9	3.9	9.7
QTL-WT-4H	Aerenchyma formation				ns	ns
QTL-WCT-4H	Aerenchyma formation				ns	ns
QTL-WCT-5H	Aerenchyma formation	5H	3257423S5	79.6	3.8	9.2
QTL-WPH-3H	Waterlogging tolerance	3H	3254867S3	104.2	17.7	36.9
QTL-WPH-4H	Waterlogging tolerance				ns	ns
QTL-WCPH-3H	Waterlogging tolerance				ns	ns
QTL-WCPH-4H	Waterlogging tolerance				ns	ns
QTL-WY-3H	Waterlogging tolerance				ns	ns
QTL-WY-4H	Waterlogging tolerance				ns	ns
QTL-WCY-4H	Waterlogging tolerance				ns	ns
QTL-WCY-7H	Waterlogging tolerance	7H	3258238S7	121.9	3.9	8.2
QTL-WT-4H	Waterlogging tolerance				ns	ns
QTL-WCT-4H	Waterlogging tolerance				ns	ns
QTL-WCT-5H	Waterlogging tolerance				ns	ns

Discussion

A new allele for aerenchyma formation from wild barley can be more effective in breeding for waterlogging tolerance in barley

A total of three QTL for waterlogging tolerance were identified from this population with all three tolerance alleles being from the wild barley. The major allele controlling waterlogging tolerance from wild barley was located on chromosome 4H at 98.8 cM on the barley physical map. This QTL was located on the same position as the QTL identified from several other populations (Zhou 2011, Zhou et al. 2012). This QTL identified from the current population explained much better phenotypic variation (34.6%) than those from other populations, including Yerong/Franklin 23.9% (Zhou 2011) and YYXT/Franklin 7.0% (Zhou et al. 2012). The minor QTL on 6H was not identified in previous reports. The minor QTL on 7H is at a similar position to that identified for leave chlorosis in the Yerong/Franklin and TX9425/Franklin populations (Li et al. 2008). However, this QTL from the Yerong/Franklin population became nonsignificant after further long term waterlogging treatment (Zhou 2011). A QTL for waterlogging tolerance was reported at 125 cM on 7H from a Chinese landrace (Xu et al. 2012), which is far away from the QTL identified in this study (71 cM on chromosome 7H).

A high resolution map of chromosome 4H provided enough markers for further marker assisted selection to improve waterlogging tolerance in barley. The location of QTL on chromosome 4H is the main region controlling waterlogging tolerance in barley and 58 candidate genes have been identified. Among all the identified 58 candidate genes, the NAC domain transcription factor and glutathione-S-transferase genes were also the candidate genes identified for lysigenous aerenchyma formation in maize (Rajhi et al. 2011, Zhang et al. 2016b). The sequence of nearest marker (3255355S4) for aerenchyma formation on chromosome 4H was used to search for barley genome sequences using NCBI blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Results suggested the gene MIR171_1 is the possible candidate gene for aerenchyma formation and waterlogging tolerance in barley. MIR171 was invovled in the regulation of metabolic adaptations to the waterlogging condititions in maize (Zhang et al. 2008). Further experiments will be required to confirm the genes for aerenchyma formation and waterlogging tolerance in barley. The region on

chromosome 4H controlling aerenchyma formation identified from the population of Franklin/TAM407227 is at the same position as that from both Yerong/Franklin and YYXT/Franklin populations (Zhou 2011, Zhou et al. 2012). The allele originating from wild barley TAM407227 not only exhibited a higher percentage of phenotypic variation (76.8% in TAM407227 vs 44% in Yerong (Zhang et al. 2016c) and 39% in YYXT (Broughton et al. 2015)), but also made a much greater contribution to waterlogging tolerance than the allele from cultivated barley varieties. Of the total percentage of phenotypic variation determined by three significant QTL (46.2%, Table 3), the allele on 4H contributed 34.6% (75% of total contribution). In contrast, the allele from Yerong contributed 23.9% to the overall waterlogging tolerance and 42% of all the contributions by four QTL (Zhou 2011). The allele from YYXT contributed only 5.2% to the overall waterlogging tolerance, which is only 11% of all the contributions by four QTL (Zhou et al. 2012). Together this further confirms that aerenchyma formation is one of the most effective mechanisms for waterlogging tolerance (Armstrong 1979). However, some of the waterlogging tolerant DH lines did not form large amounts of aerenchyma, indicating the possible existence of other mechanisms involved in waterlogging tolerance, such as development of adventitious roots (Mano et al. 2005), formation of the barrier to radial oxygen loss (Colmer and Voesenek 2009), or increased tolerance to elemental or metabolite toxicity (Shabala et al. 2014). These mechanisms play more important roles in waterlogging tolerance in cultivated barley, while in wild barley TAM407227 the allele controlling aerenchyma formation was shown to be most effective in improving waterlogging tolerance, thus can be effectively used in future breeding programs. Similar results have been reported in other crops. Wild relatives of maize are able to form aerenchyma without waterlogging stress (Mano et al. 2006). Wild relatives of wheat showed higher root porosity and lower radial oxygen loss under waterlogging conditions (Malik et al. 2009). These favourable traits of waterlogging tolerance in wild relatives of maize and wheat have been successfully transferred to cultivated maize and wheat (Malik et al. 2011, Mano and Omori 2013).

Selecting for waterlogging tolerance

Higher yield under waterlogging stress is always an important target in plant breeding. However, the heritability of yield under waterlogging conditions is relatively low (Collaku and Harrison 2005) and therefore difficult to be directly used in breeding programs. The genes contributing to high yield under abiotic stresses might be the same as those controlling

higher yield under well drained conditions with nothing to do with stress tolerance (Jones 2007). Thus relative changes in different paremeters (stressed/control) are always used as indicators for stress tolerance. QTL for yield and other useful agronomic traits (plant height and tiller number) under control and waterlogging conditions were also identified. Two QTL for WPH and WCPH were identified at the same positions on chromosomes 3H and 4H. A QTL for CPH was identified at the same position of a QTL for WPH and WCPH on chromosome 3H. When aerenchyma formation and waterlogging tolerance were used as covariates, QTL for WCPH became insignificant. However, the QTL for WPH on chromosome 3H was not affected by aerenchyma formation and waterlogging tolerance. This suggested the importance of using relative changes in different paremeters (stressed/control) as indicators for waterlogging tolerance.

In this work the correlation coefficient between waterlogging tolerance and WY was the highest among all the traits ($r = 0.7$) and the QTL for WY and WCY on chromosome 4H is the same position of QTL for waterlogging tolerance based on plant survival. The QTL for WY and WCY on chromosome 4H could not be detected when aerenchyma formation and waterlogging tolerance were used as covariates. This further confirmed the effectiveness of using aerenchyma formation and waterlogging tolerance as the selection criteria to improve the yield under waterlogging conditions. The QTL for waterlogging tolerance on chromosome 7H is in the same position as the QTL for quantum yield under hypoxia in barley (Bertholdsson et al. 2015, Zhang et al. 2016b).

Plant architecture traits, such as plant height and tillers, are reported to be possible target traits to improve yield (Khush 2001). A QTL on chromosome 3H at around 110 cM was identified for CPH, WPH, WCPH, CY and WY. This region also controls drought tolerant QTL, such as plant height and peduncle length under drought stress (Korff et al. 2008, Zhang et al. 2016b). QTL analysis for CY using CPH as a covariate suggested that plant height did not have a significant effect on yield. This position on chromosome 3H provided a useful resource for breeders to improve yield of barley.

In conclusion, a new allele for aerenchyma formation under waterlogging stress was identified from a wild barley accession. This allele showed a much better ability in forming aerenchyma and was the major contributor to waterlogging tolerance. A high density linkage

map helped identify several co-segregating markers that can be directly used in breeding programs.

Chapter 6: Meta-analysis of major QTL for abiotic stress tolerance in barley

Abstract

Drought, salinity and waterlogging are three major abiotic stresses limiting barley yield world-wide. Breeding for abiotic stress tolerant crops has drawn increased attention and a large number of quantitative trait loci (QTL) for drought, salinity, and waterlogging tolerance in barley have been detected. However, very few QTL have been successfully used in marker assisted selection (MAS) in breeding. In this study, we summarized 632 QTL for drought, salinity and waterlogging tolerance in barley. Among all these QTL, only 195 major QTL were used to conduct meta-analysis to refine QTL positions for MAS. Meta-analysis was used to map the summarized major QTL for drought, salinity, and waterlogging tolerance from different mapping populations on the barley physical map. The positions of identified meta-QTL (MQTL) were used to search for candidate genes for drought, salinity, and waterlogging tolerance in barley. Both MQTL3H.4 and MQTL6H.2 control drought tolerance in barley. Fine mapped QTL for salinity tolerance, *HvNax4* and *HvNax3*, were validated on MQTL1H.4 and MQTL7H.2, respectively. MQTL2H.1 and MQTL5H.3 were also the target regions for improving salinity tolerance in barley. MQTL4H.4 is the main region controlling waterlogging tolerance in barley with fine mapped QTL for aerenchyma formation under waterlogging conditions. Detected and refined MQTL and candidate genes are crucial for future successful MAS in barley breeding.

Introduction

Continued crop improvement is of paramount importance for feeding an increasing human population. Global breeding efforts over the past century have made significant contributions

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to increased yield potential and stability, as well as cultivars with more durable levels of tolerance to a diverse array of abiotic (drought, freezing, salinity and waterlogging) stresses (Khush 2001). Breeding crops that are tolerant to abiotic stresses is still the best approach to increase crop production (Gill and Tuteja 2010, Tester and Langridge 2010).

Quantitative trait loci (QTL) analysis is a powerful tool in agriculture and other fields. It provides knowledge of the chromosomal location of the target loci and can be applied in breeding programs using marker assisted selection (MAS). Molecular markers linked to specific QTL have provided plant breeders with a method to improve selecting desirable recombinants from superior varieties and accelerating breeding programs (Khush 2001). MAS, combined with conventional breeding, has been utilized in many parts of the world and on many crops (Singh, Mackill and Ismail 2009).

The number of publications reporting the identification of new QTL has been increasing tremendously during the past two decades, involving many crop plants and all types of agronomic traits (Xu and Crouch 2008). However, reports of QTL mapping to date are mostly based on a relatively low amount of markers, providing limited marker–trait association; and few of the QTL reported have been efficiently used for MAS in plant breeding (William, Trethowan and Crosby-Galvan 2007). Many QTL could be identified for one trait, but most of them explain a small proportion of phenotypic variances of the traits (Tuberosa 2012). Therefore, plant breeding programs have not been able to take full advantage of these QTL (Eagles et al. 2001, Xu and Crouch 2008). Positional cloning (DNA sequence identification) of the QTL that explain more than 15% phenotypic variance can greatly increase the effectiveness of using MAS in breeding programs (Salvi and Tuberosa 2005).

Drought, salinity and waterlogging are three major abiotic stresses limiting the yield of crops, causing extensive losses worldwide (Mittler 2006, Qin, Shinozaki and Yamaguchi-Shinozaki 2011). Numerous QTL for drought, salinity and waterlogging tolerance in barley have been described. A meta-analysis can be used to combine different experimental results in one single study. At the QTL level, meta-analysis is able to map the QTL on the same linkage group from different mapping populations of different traits and lower the confidence of interval of QTL to identify more effective candidate genes (Goffinet and Gerber 2000). So far, meta-analysis has been successfully used in studying QTL for flowering time in maize

(Chardon et al. 2004, Wang et al. 2016b), drought tolerance in rice (Khowaja et al. 2009), agronomic traits in cotton (Said et al. 2015), leaf senescence in *Arabidopsis* (Chardon et al. 2014) and yield related traits in wheat (Zhang et al. 2010).

In this study, we summarized 632 QTL for drought, salinity and waterlogging tolerance in barley. Among all these QTL, only 195 major QTL were used to perform meta-analysis to refine QTL positions for MAS. We also identified candidate genes for each of the meta-QTL. Identified meta-QTL from meta-analysis provide resources for further MAS and various omics studies.

Materials and methods

Development of databases

Overall, 632 QTL identified from 1994 to 2015 for drought, salinity and waterlogging tolerance from 32 peer-reviewed publications were summarized in barley. Each QTL represents QTL for different traits from different studies with some of them being located in similar positions. Major QTL with the LOD value above 3 and the value of phenotypic variance exceeding 10% were selected for the meta-analysis, as only QTL with these qualities can potentially be used in MAS and positional cloning (Collard et al. 2005). Parameters under control conditions are able to provide the tolerance coefficients caused by stresses with the relative changes of parameters (stressed/control). Therefore, many QTL for the tolerance coefficients and QTL for traits under control conditions are reported. We included QTL for the tolerance coefficients, but excluded QTL for the traits under control conditions. Although plant architecture traits, such as reduced height, increased number of tillers and erect leaves were reported to be also effective in breeding under control conditions (Khush 2001), these QTL are not relevant to the present investigation. Therefore, we reduced the number of QTL to 195 (Table 6.1). All of these 195 major QTL were used for meta-analysis.

Table 6.1 Summary of the major QTL for abiotic stress tolerance in barley.

F0 is initial fluorescence, Fm is maximum fluorescence, Fv is variable fluorescence, and PSII (Fv/Fm) is maximum quantum efficiency
WSC is water soluble carbohydrate, RWC is relative water content

Original QTL	Original QTL name	Stresses	Location	MQTL	LOD score	Phenotypic variance	Position	From	To	Reference	Parents
QHSFW.1H	Hypoxia shoot fresh weight	Waterlogging	1H	MQTL1H.1	3.68	16	3.27	0	12.9	Broughton et al. 2015	Franklin / YYXT
QSlww.YG.1H-1	Combined salinity and waterlogging tolerance winter	Combined salinity and waterlogging	1H	MQTL1H.1	5.99	15.4	10.3	0.47	20.13	Ma et al. 2015	YSM1/Gairdner
QHLRL.1H	Hypoxia longest root length	Waterlogging	1H	MQTL1H.2	3.69	11.2	36.62	22.9	50.38	Broughton et al. 2015	Franklin / YYXT
QHSDW.1H	Hypoxia shoot dry weight	Waterlogging	1H	MQTL1H.2	3.35	11.5	37	23.6	50.4	Broughton et al. 2015	Franklin / YYXT
QGDmin	Drought minimum germination rate	Drought	1H	MQTL1H.2	7.25	49.5	38	32.9	43.1	Zhang et al. 2005	Mona / *Hordeum spontaneum*
QSC.1H	Salt tolerance	Salinity	1H	MQTL1H.2	5.2	16.2	45.5	40	51	Mano and	Steptoe / Morex

	at seedling stage									Tekeda 1997	
QHRFW.1H	Hypoxia root fresh weight	Waterlogging	1H	MQTL1H.2	3.37	10.3	50	35	64.96	Broughton et al. 2015	Franklin / YYXT
QHRDW.1H	Hypoxia root dry weight	Waterlogging	1H	MQTL1H.2	3.17	11	50	36	64.01	Broughton et al. 2015	Franklin / YYXT
QTL.SRK	Salinity root K+	Salinity	1H	MQTL1H.3	3.64	10.1	51	33.5	68.49	Nguyen et al. 2013	Steptoe / Morex
QCh1Hb	Salinity chlorophyll content	Salinity	1H	MQTL1H.3	4.57	18.67	55	35.3	74.71	Siahsar and Aminfar 2010	Steptoe / Morex
QSB.1H	Salt tolerance at seedling stage	Salinity	1H	MQTL1H.3	3	10.5	58	55	61	Mano and Tekeda 1997	Steptoe / Morex
Qsl-tera_1H.a	Drought spike length	Drought	1H	MQTL1H.3	3.406	10.6	68	52.2	83.82	Korff et al. 2008	Tadmor / ER/Apm
QSA.1H	Salt tolerance at seedling stage	Salinity	1H	MQTL1H.3	4.5	16.4	70	61	79	Mano and Tekeda 1997	Steptoe / Morex
QSlww.YG.1H-2	Combined salinity and waterlogging tolerance winter	Combined salinity and waterlogging	1H	MQTL1H.3	4.66	11.5	72.2	59	85.37	Ma et al. 2015	YSM1/Gairdner
Qdh-tera_1H.b	Drought days of heading	Drought	1H	MQTL1H.4	4.512	15.8	101	90.4	111.6	Korff et al. 2008	Tadmor / ER/Apm
Qgv-tera_1H.a	Drought early growth vigour	Drought	1H	MQTL1H.4	4.23	20.8	101	92.9	109.1	Korff et al. 2008	Tadmor / ER/Apm
Qdm-tera_1H.a	Drought days of maturity	Drought	1H	MQTL1H.4	6.811	36.9	101	96.5	105.5	Korff et al. 2008	Tadmor / ER/Apm
Qfp-tera_1H.a	Drought grain filling period	Drought	1H	MQTL1H.4	5.488	42.6	101	97.1	104.9	Korff et al. 2008	Tadmor / ER/Apm
HvNax4	Salinity shoot Na+	Salinity	1H	MQTL1H.4	39.1	84	102.5	101	104	Rivandi et al. 2011	Clipper / Sahara 3771

	concentration										
QSG.1H	Salt tolerance germination	Salinity	1H	MQTL1H.4	5.5	17.4	105	90	120	Mano and Tekeda 1997	Harrington / TR306
QWS.S42.1H	Drought wilting score	Drought	1H	MQTL1H.4	3.4	12	109	94.9	123.1	Sayed et al. 2012	Scarlett / ISR42-8
QSlsd.YG.1H	Salinity tolerance summer	Salinity	1H	MQTL1H.5	7.06	16	129.8	120	133	Ma et al. 2015	YSM1/Gairdner
QRDmin.2H	Drought minimum revival rate	Drought	2H	MQTL2H.1	9.02	66.9	3.1	0	6.5	Zhang et al. 2005	Mona / *Hordeum spontaneum*
QPr2H	Salinity proline content	Salinity	2H	MQTL2H.1	3.92	13.73	4	0	30.81	Siahsar and Aminfar 2010	Steptoe / Morex
QRWC2H	Salinity relative water content	Salinity	2H	MQTL2H.1	3.6	14.92	4	0	28.67	Siahsar and Aminfar 2010	Steptoe / Morex
tfy1.1-1	Waterlogging leaf chlorosis 2 weeks 2004	Waterlogging	2H	Not projected	9.21	23.3	4.315	3.6	5.03	Li et al. 2008	Franklin / TX9425
QSl.TxNn.2H	Salinity tolerance score	Salinity	2H	MQTL2H.1	24.37	45	16.61	14.4	18.81	Xu et al. 2012	TX9425 / Naso Nijo
QGS.2H	Salt germination speed	Salinity	2H	MQTL2H.1	6.4	18	18.5	15	22	Mano and Tekeda 1997	Steptoe / Morex
QSRD.TxNn.2H	Salinity root dry weight	Salinity	2H	MQTL2H.1	12.42	23.2	18.81	12.7	24.89	Xu et al. 2012	TX9425 / Naso Nijo
QSRF.TxNn.2H	Salinity root fresh weight	Salinity	2H	MQTL2H.1	10.99	23.6	18.81	12.8	24.78	Xu et al. 2012	TX9425 / Naso Nijo
QTL.SSK	Salinity shoot K+	Salinity	2H	MQTL2H.1	6.81	18.8	22	12.6	31.4	Nguyen et al. 2013	Steptoe / Morex
QTL.SSCl.2H	Salinity shoot Cl-	Salinity	2H	MQTL2H.1	9.8	23.8	22	14.6	29.42	Nguyen et al. 2013	Steptoe / Morex

QTL.SSNa	Salinity shoot Na+	Salinity	2H	MQTL2H.1	9.82	23.8	22	14.6	29.42	Nguyen et al. 2013	Steptoe / Morex
QWl.TxNn.2H	Waterlogging tolerance score (9 weeks)	Waterlogging	2H	MQTL2H.1	9.93	16	22.42	18.8	26.03	Xu et al. 2012	TX9425 / Naso Nijo
QTL.SSFWR	Salinity shoot fresh weight reduction	Salinity	2H	MQTL2H.1	3.05	10.2	23	5.68	40.32	Nguyen et al. 2013	Steptoe / Morex
QTL.SRDW	Salinity root dry weight	Salinity	2H	MQTL2H.1	5.71	17.9	23	13.1	32.87	Nguyen et al. 2013	Steptoe / Morex
QSY.TxNn.2H	Salinity number of yellow leaves	Salinity	2H	MQTL2H.1	7.36	15.1	25.73	16.4	35.06	Xu et al. 2012	TX9425 / Naso Nijo
QSl.YyFr.2H	Salinity tolerance score	Salinity	2H	MQTL2H.1	7.64	10.6	26	23	29	Zhou et al. 2012a	Franklin / YYXT
QSD.TxNn.2H	Salinity green leaves dry weight	Salinity	2H	MQTL2H.1	9.17	18.1	27.07	7.76	46.38	Xu et al. 2012	TX9425 / Naso Nijo
QSL.TxNn.2H	Salinity green leaves fresh weight	Salinity	2H	MQTL2H.1	4.39	10.5	27.07	13.6	40.49	Xu et al. 2012	TX9425 / Naso Nijo
QSRFP.TxNn.2H	Salinity root fresh weight per plant	Salinity	2H	MQTL2H.1	4.58	10.6	27.07	13.8	40.37	Xu et al. 2012	TX9425 / Naso Nijo
QRLE.2H	Drought root length	Drought	2H	MQTL2H.1	7.8	14.3	29	15.2	42.83	Chen et al. 2010	WQ23-38 / MA10-30
QRWC.2H	Drought relative water content	Drought	2H	MQTL2H.1	3.28	25.1	29	21.1	36.88	Chen et al. 2010	WQ23-38 / MA10-30
QSlsw.YG.2H	Combined salinity and	Combined salinity and	2H	MQTL2H.1	3.7	10.2	31.5	16.7	46.35	Ma et al. 2015	YSM1/Gairdner

	waterlogging tolerance summer	waterlogging									
QSB.2H.1	Salt tolerance at seedling stage	Salinity	2H	MQTL2H.2	4.4	13.7	34	28	40	Mano and Tekeda 1997	Steptoe / Morex
QDT.TxFr.2H	Drought tolerance score	Drought	2H	MQTL2H.2	8.56	42.2	35.81	27.1	44.53	Fan et al. 2015	TX9425/Franklin
QRMO.TxFr.2H	Drought relative water content	Drought	2H	MQTL2H.2	9.45	45.4	38.97	30.9	47.08	Fan et al. 2015	TX9425/Franklin
QSlsd.YG.2H	Salinity tolerance summer	Salinity	2H	MQTL2H.2	7.78	17.9	39.5	31	47.96	Ma et al. 2015	YSM1/Gairdner
QRER.2H	Drought leaf relative elongation rate	Drought	2H	MQTL2H.2	3.73	52.6	46	42.2	49.76	Chen et al. 2010	WQ23-38 / MA10-30
QSA.2H.1	Salt germination ABA	Salinity	2H	MQTL2H.2	3.4	12.1	51	46	56	Mano and Tekeda 1997	Steptoe / Morex
Qdh-tera_2H.a	Drought days of heading	Drought	2H	MQTL2H.3	3.839	11.1	57	41.9	72.11	Korff et al. 2008	Tadmor / ER/Apm
WL4.1	Waterlogging tolerance score (4 weeks)	Waterlogging	2H	MQTL2H.3	5.7	10.2	64.17	46.3	81.99	Zhou 2011	Franklin / Yerong
QWl.YyFr.2H	Waterlogging tolerance score (9 weeks)	Waterlogging	2H	MQTL2H.3	18.68	30.1	64.17	59.1	69.29	Zhou et al. 2012c	Franklin / YYXT
QDC.2H	Drought water-soluble	Drought	2H	MQTL2H.4	3.02	18.6	77	69.4	84.62	Teulat et al. 2001	Tadmor / ER/Apm

	carbohydrate concentration										
KWw2.1	Waterlogging kernel weight 06-07	Waterlogging	2H	MQTL2H.4	9.1	27.35	81.9	81.7	82.1	Xue et al. 2010	Franklin / Yerong
SLw2.1	Waterlogging spike length 06-07	Waterlogging	2H	MQTL2H.4	11.16	17.44	81.9	81.7	82.1	Xue et al. 2010	Franklin / Yerong
GSw1.1	Waterlogging grains per spike 05-06	Waterlogging	2H	MQTL2H.4	11.53	35.35	82.13	77.3	86.94	Xue et al. 2010	Franklin / Yerong
GSw2.1	Waterlogging grains per spike 06-07	Waterlogging	2H	MQTL2H.4	5.63	55.34	82.13	79.1	85.2	Xue et al. 2010	Franklin / Yerong
QCCW.2H	Drought the contribution of a change in water content to osmotic adjustment content to OA	Drought	2H	MQTL2H.4	5.6	11.9	84	72.1	95.91	Teulat et al. 2001	Tadmor / ER/Apm
qGNP2s	Salinity grain number per plant	Salinity	2H	MQTL2H.4	6.83	25.33	84.4	73.2	95.65	Xue et al. 2009	CM72 / Gairdner
tfsur-1	Waterlogging plant survival	Waterlogging	2H	MQTL2H.4	3.29	19	92.04	81.7	102.4	Li et al. 2008	Franklin / TX9425
GSw1.2	Waterlogging grains per spike 05-06	Waterlogging	2H	Not projected	6.29	12.18	108.5	108	108.7	Xue et al. 2010	Franklin / Yerong
KWw1.2	Waterlogging kernel weight 05-06	Waterlogging	2H	Not projected	7.34	16.59	108.5	108	108.7	Xue et al. 2010	Franklin / Yerong
SLw2.2	Waterlogging spike length	Waterlogging	2H	MQTL2H.5	8.74	13.05	114.4	101	127.4	Xue et al. 2010	Franklin / Yerong

QtFo2.1	06-07 Drought F0	Drought	2H	MQTL2H.5	3.9	13.5	119	109	129.1	Guo et al. 2008	Arta / Hordeum spontaneum 41-1
QtFm2.1	Drought Fm	Drought	2H	MQTL2H.5	5.4	15.1	119	110	128	Guo et al. 2008	Arta / Hordeum spontaneum 41-1
QtFv/Fm2.1	Drought Fv/Fm	Drought	2H	MQTL2H.5	5.2	15.5	119	110	127.8	Guo et al. 2008	Arta / Hordeum spontaneum 41-1
QtFv2.1	Drought Fv	Drought	2H	MQTL2H.5	5.7	16.3	119	111	127.4	Guo et al. 2008	Arta / Hordeum spontaneum 41-1
QWL.YeFr.2H.2	Waterlogging tolerance score (9 weeks)	Waterlogging	2H	MQTL2H.5	10.21	17.2	129.1	120	137.8	Zhou 2011	Franklin / Yerong
QWSC2H	Salinity water soluble carbohydrate	Salinity	2H	MQTL2H.5	3.73	20.51	133	115	150.9	Siahsar and Aminfar 2010	Steptoe / Morex
QFmv2H	Salinity Fv/Fm	Salinity	2H	MQTL2H.5	13.91	24.81	133	118	147.8	Siahsar and Aminfar 2010	Steptoe / Morex
QFv2H	Salinity Fv	Salinity	2H	MQTL2H.5	12.88	44.69	133	125	141.2	Siahsar and Aminfar 2010	Steptoe / Morex
WL5.3	Waterlogging tolerance score (5 weeks)	Waterlogging	2H	MQTL2H.5	6.79	11.3	138.2	123	153.5	Zhou 2011	Franklin / Yerong
QHLRL.2H	Hypoxia longest root length	Waterlogging	2H	MQTL2H.6	3.53	10.7	156.4	142	170.8	Broughton et al. 2015	Franklin / YYXT
QWSC100S.2H.3	Drought water-soluble carbohydrate concentration at full turgor	Drought	2H	MQTL2H.6	4.8	11	181	167	181	Diab et al. 2004	Tadmor / ER/Apm

QWSCS.2H	Drought water-soluble carbohydrate concentration	Drought	2H	MQTL2H.6	4.8	15	181	170	181	Diab et al. 2004	Tadmor / ER/Apm
QL2L.2H	Drought second leaf length	Drought	2H	MQTL2H.6	3.02	32.9	181	175	181	Chen et al. 2010	WQ23-38 / MA10-30
QDWSC100.3H.1	Drought accumulation of WSC at 100%RWC	Drought	3H	MQTL3H.1	4.5	10	0	0	19.87	Diab et al. 2004	Tadmor / ER/Apm
QRDmin.3H	Drought minimum revival rate	Drought	3H	MQTL3H.1	4.14	17.2	4.4	0	13.5	Zhang et al. 2005	Mona / *Hordeum spontaneum*
QTL.Na/K	Salinity Na+/K+ ratio	Salinity	3H	MQTL3H.1	3.6	11.2	15	0	30.77	Nguyen et al. 2013	Steptoe / Morex
QWl.YyFr.3H	Waterlogging tolerance score (9 weeks)	Waterlogging	3H	MQTL3H.1	10.94	15.7	10.72	1.48	19.95	Zhou et al. 2012c	Franklin / YYXT
QTL.SSNa/K.3H	Salinity shoot Na+/K+ ratio	Salinity	3H	MQTL3H.1	5.28	14.7	15	2.98	27.02	Nguyen et al. 2013	Steptoe / Morex
QHTiller.3H	Hypoxia tiller number	Waterlogging	3H	MQTL3H.2	5.63	16.7	35.93	26.7	45.16	Broughton et al. 2015	Franklin / YYXT
QREG.3H	Drought regrowth rate	Drought	3H	MQTL3H.2	3.47	19	51	40.6	61.41	Chen et al. 2010	WQ23-38 / MA10-30
QFv3H	Salinity Fv	Salinity	3H	MQTL3H.2	6.52	28.88	60	47.3	72.74	Siahsar and Aminfar 2010	Steptoe / Morex
yfy2.1-2	Waterlogging leaf yellowing 2 weeks 2005	Waterlogging	3H	MQTL3H.2	6.41	11.9	59.05	51.6	66.5	Li et al. 2008	Franklin / Yerong
Qped-tera_3H.b	Drought	Drought	3H	MQTL3H.3	6.139	13.5	75	62.6	87.42	Korff et al.	Tadmor /

	peduncle length									2008	ER/Apm
Qpedex-tera_3H.b	Drought peduncle extrusion	Drought	3H	MQTL3H.3	4.469	16.3	75	64.7	85.29	Korff et al. 2008	Tadmor / ER/Apm
QPC-S.TxFr.3H	Salinity proline contents	Salinity	3H	MQTL3H.3	3.22	18.6	84.89	65.1	104.7	Fan et al. 2015	TX9425/Franklin
Qph-tera_3H.b	Drought plant height	Drought	3H	MQTL3H.3	5.184	18.6	75	66	84.02	Korff et al. 2008	Tadmor / ER/Apm
QPC-D.TxFr.3H	Drought proline contents	Drought	3H	MQTL3H.3	6.65	34.7	77.84	67.2	88.45	Fan et al. 2015	TX9425/Franklin
QGS.3H	Salt germination speed	Salinity	3H	MQTL3H.3	7.1	20.3	89.5	76	103	Mano and Tekeda 1997	Steptoe / Morex
tfy1.1-2	Waterlogging leaf chlorosis 2 weeks 2004	Waterlogging	3H	MQTL3H.3	7.59	33.4	87.45	84.9	90	Li et al. 2008	Franklin / TX9425
tfy2.1-1	Waterlogging leaf chlorosis 2 weeks 2005	Waterlogging	3H	MQTL3H.3	9.28	34.1	87.45	84.9	90	Li et al. 2008	Franklin / TX9425
tfy1.2-1	Waterlogging leaf chlorosis 4 weeks 2004	Waterlogging	3H	MQTL3H.3	7.31	36	87.45	84.9	90	Li et al. 2008	Franklin / TX9425
SLw2.3	Waterlogging spike length 06-07	Waterlogging	3H	MQTL3H.3	7.74	14.37	92.57	87.8	97.36	Xue et al. 2010	Franklin / Yerong
QDWSC100.3H.3	Drought accumulation of WSC at 100%RWC	Drought	3H	MQTL3H.4	3.2	11	103	88.6	117.4	Diab et al. 2004	Tadmor / ER/Apm
Qpedex-tera_3H.a	Drought peduncle	Drought	3H	MQTL3H.4	4.165	12.3	103	89.4	116.6	Korff et al. 2008	Tadmor / ER/Apm

	extrusion										
Qped-tera_3H.a	Drought peduncle length	Drought	3H	MQTL3H.4	6.833	19	103	94.2	111.8	Korff et al. 2008	Tadmor / ER/Apm
Qph-tera_3H.a	Drought plant height	Drought	3H	MQTL3H.4	4.816	19.4	103	94.4	111.6	Korff et al. 2008	Tadmor / ER/Apm
QWSC100S.3H.2	Drought water-soluble carbohydrate concentration at full turgor	Drought	3H	MQTL3H.4	5.9	26	103	96.9	109.1	Diab et al. 2004	Tadmor / ER/Apm
Qph-tera_3H.c	Drought plant height	Drought	3H	MQTL3H.4	3.753	13.6	118	106	130.3	Korff et al. 2008	Tadmor / ER/Apm
Qpedex-tera_3H.c	Drought peduncle extrusion	Drought	3H	MQTL3H.4	3.601	14.2	118	106	129.8	Korff et al. 2008	Tadmor / ER/Apm
Qped-tera_3H.c	Drought peduncle length	Drought	3H	MQTL3H.4	3.102	16.5	118	108	128.2	Korff et al. 2008	Tadmor / ER/Apm
QWS.S42.3H	Drought wilting score	Drought	3H	MQTL3H.5	5	34	130.3	119	141.9	Sayed et al. 2012	Scarlett / ISR42-8
qPH3s	Salinity plant height	Salinity	3H	MQTL3H.5	5.17	14.15	145.4	143	148	Xue et al. 2009	CM72 / Gairdner
QSG.4H	Salt tolerance germination	Salinity	4H	MQTL4H.1	5.1	14.7	0.5	0	1	Mano and Tekeda 1997	Steptoe / Morex
QRDs	Drought slopes of revival rate	Drought	4H	MQTL4H.1	19.2	68.3	6.1	2	10.2	Zhang et al. 2005	Mona / Hordeum spontaneum
QHTiller.4H	Hypoxia tiller number	Waterlogging	4H	MQTL4H.1	4.8	14	14.6	3.6	25.6	Broughton et al. 2015	Franklin / YYXT
QTL.SRS	Salinity root SO42-	Salinity	4H	MQTL4H.2	3.14	10.3	47	29.8	64.15	Nguyen et al. 2013	Steptoe / Morex
QREG.4H	Drought	Drought	4H	MQTL4H.2	4.3	34.3	53	47.2	58.77	Chen et al.	WQ23-38 /

	regrowth rate									2010	MA10-30
qSPL4s	Salinity spikes per line	Salinity	4H	MQTL4H.3	10.57	27.3	66.75	65.6	67.92	Xue et al. 2009	CM72 / Gairdner
QPSII.sthf-4H.2	Drought (Fm–Fs)/Fm	Drought	4H	MQTL4H.4	5.26	13.85	86.69	76.2	97.15	Wojcik-Jagla et al. 2013	MOB12055 / Suweren
Qqp.sthf-4H.2	Drought qP = (Fm–Fs)/(Fm–F0)	Drought	4H	MQTL4H.4	5.81	15.13	86.69	77.1	96.26	Wojcik-Jagla et al. 2013	MOB12055 / Suweren
WL3.4	Waterlogging tolerance score (3 weeks)	Waterlogging	4H	MQTL4H.4	10.01	15.8	91	81.5	100.5	Zhou 2011	Franklin / Yerong
QCCW.4H	Drought the contribution of a change in water the contribution of a change in water content to OA	Drought	4H	MQTL4H.4	3.11	12.4	99	87.6	110.4	Teulat et al. 2001	Tadmor / ER/Apm
QNSOA.4H	Drought net solute accumulation to osmotic adjustment	Drought	4H	MQTL4H.4	3.38	14.1	99	88.9	109.1	Teulat et al. 2001	Tadmor / ER/Apm
QHPorosity.4H	Hypoxia root porosity	Waterlogging	4H	MQTL4H.4	13.52	39	97	93	101	Broughton et al. 2015	Franklin / YYXT
WL4.3	Waterlogging tolerance score (4 weeks)	Waterlogging	4H	MQTL4H.4	10.02	17.7	104	95.5	112.4	Zhou 2011	Franklin / Yerong

yfy2.1-3	Waterlogging leaf yellowing 2 weeks 2005	Waterlogging	4H	MQTL4H.4	9.25	18.5	104	95.9	112	Li et al. 2008	Franklin / Yerong
yfy2.2-3	Waterlogging leaf yellowing 4 weeks 2005	Waterlogging	4H	MQTL4H.4	10.37	22.4	104	97.3	110.6	Li et al. 2008	Franklin / Yerong
QTL-rp4H	Waterlogging root porosity	Waterlogging	4H	MQTL4H.4	8.12	26.2	98.55	97.7	99.43	Zhang et al. 2016	Franklin / Yerong
QTL-aerenchyma	Waterlogging aerenchyma formation	Waterlogging	4H	MQTL4H.4	21.2	42.8	98.55	97.7	99.43	Zhang et al. 2016	Franklin / Yerong
QWL.YeFr.4H	Waterlogging tolerance score (9 weeks)	Waterlogging	4H	MQTL4H.4	14.84	23.9	104	97.7	110.2	Zhou 2011	Franklin / Yerong
WL5.4	Waterlogging tolerance score (5 weeks)	Waterlogging	4H	MQTL4H.4	16.51	26.7	104	98.3	109.6	Zhou 2011	Franklin / Yerong
QWSC100S.4H	Drought water-soluble carbohydrate concentration at full turgor	Drought	4H	MQTL4H.5	7.9	12	117	104	117	Diab et al. 2004	Tadmor / ER/Apm
QDWSC100.4H	Drought accumulation of WSC at 100%RWC	Drought	4H	MQTL4H.5	6.5	13	117	105	117	Diab et al. 2004	Tadmor / ER/Apm
QGS.5H.3	Salt germination speed	Salinity	5H	MQTL5H.1	3.7	11.7	5	1	9	Mano and Tekeda 1997	Harrington / TR312
QDOP.5H	Drought leaf osmotic potential	Drought	5H	MQTL5H.1	3.58	20	9	1.91	16.09	Teulat et al. 2001	Tadmor / ER/Apm

QWC.sthf-5H.3	Drought water content	Drought	5H	MQTL5H.2	3.25	11.36	43	30.3	55.75	Wojcik-Jagla et al. 2013	MOB12055 / Suweren
QFo5Ha	Salinity F0	Salinity	5H	MQTL5H.3	3.73	15.51	50	26.3	73.73	Siahsar and Aminfar 2010	Steptoe / Morex
QSG.5H	Salt tolerance germination	Salinity	5H	MQTL5H.2	19.4	46.7	50.5	50	51	Mano and Tekeda 1997	Steptoe / Morex
QRW.5H	Salinity root dry weight	Salinity	5H	MQTL5H.3	7	10	56	52	60	Ellis et al. 2002	Derkado / B83-12/21/5
QSW.5H	Salinity shoot dry weight	Salinity	5H	MQTL5H.3	6.6	10	56	52	60	Ellis et al. 2002	Derkado / B83-12/21/5
QC.5H	Salinity shoot total C concentration	Salinity	5H	MQTL5H.3	10.3	15	56	52	60	Ellis et al. 2002	Derkado / B83-12/21/5
QSA.5H.1	Salt germination ABA	Salinity	5H	MQTL5H.3	15.6	43.3	56.5	51	62	Mano and Tekeda 1997	Steptoe / Morex
QSG.5H	Salinity germination	Salinity	5H	MQTL5H.3	10.14	42	56.5	51	62	Witzel el al. 2010	DOM / REC
QSl.YyFr.5H	Salinity tolerance score	Salinity	5H	MQTL5H.4	7.38	10.3	71.95	57	86.91	Zhou et al. 2012a	Franklin / YYXT
QRLE.5H	Drought root length	Drought	5H	MQTL5H.4	3.85	30.4	72	65.5	78.51	Chen et al. 2010	WQ23-38 / MA10-30
yfsur-2	Waterlogging plant survival	Waterlogging	5H	MQTL5H.5	5.05	13.1	92.29	86.3	98.23	Li et al. 2008	Franklin / Yerong
GSw2.3	Waterlogging grains per spike 06-07	Waterlogging	5H	MQTL5H.5	6.18	10.02	97	97.9	97.9	Xue et al. 2010	Franklin / Yerong
QDI.sthm-5H	Drought dissipated from PSII (DIo/CS)	Drought	5H	MQTL5H.5	3.07	10.84	98.23	84.9	111.6	Wojcik-Jagla et al. 2013	STH836 / STH758
QDWSC100.5H	Drought accumulation	Drought	5H	MQTL5H.5	4.2	12	99	85.8	112.2	Diab et al. 2004	Tadmor / ER/Apm

	of WSC at 100%RWC										
QDWC.5H.2	Drought relative water content	Drought	5H	MQTL5H.5	4.42	11.5	111	97.2	124.8	Teulat et al. 2003	Tadmor / ER/Apm
QSlsw.YG.5H	Combined salinity and waterlogging tolerance summer	Combined salinity and waterlogging	5H	MQTL5H.6	6.31	18.4	120.9	113	129.1	Ma et al. 2015	YSM1/Gairdner
QDT.TxFr.5H	Drought tolerance score	Drought	5H	MQTL5H.7	4.13	14	125.7	99.4	152	Fan et al. 2015	TX9425/Franklin
QSA.5H	Salt tolerance at seedling stage	Salinity	5H	MQTL5H.6	4	13.8	127.5	126	129	Mano and Tekeda 1997	Harrington / TR313
QSB.5H	Salt tolerance at seedling stage	Salinity	5H	MQTL5H.6	5.4	17.4	127.5	126	129	Mano and Tekeda 1997	Harrington / TR314
QNaKsd.5H	Salinity Na+/K+ ratio	Salinity	5H	MQTL5H.7	5.62	16	130.9	121	140.4	Ma et al. 2015	YSM1/Gairdner
QSA.5H	Salt tolerance at seedling stage	Salinity	5H	MQTL5H.7	3.9	11.8	132.5	130	135	Mano and Tekeda 1997	Steptoe / Morex
QHRFW.5H	Hypoxia root fresh weight	Waterlogging	5H	MQTL5H.7	4.31	13.4	132.9	121	144.4	Broughton et al. 2015	Franklin / YYXT
QL2L.5H	Drought second leaf length	Drought	5H	MQTL5H.7	6.54	52.2	137	133	140.8	Chen et al. 2010	WQ23-38 / MA10-30
QTL.SSP	Salinity shoot PO43-	Salinity	5H	MQTL5H.7	6.33	17.9	138	128	147.9	Nguyen et al. 2013	Steptoe / Morex
QSC.5H	Salt tolerance at seedling stage	Salinity	5H	MQTL5H.7	9.4	30.8	139.5	135	144	Mano and Tekeda 1997	Harrington / TR315

QPr5Hb	Salinity proline content	Salinity	5H	MQTL5H.7	4	18.28	150	130	170.1	Siahsar and Aminfar 2010	Steptoe / Morex
QFv5H	Salinity Fv	Salinity	5H	MQTL5H.7	4.75	19.15	150	131	169.2	Siahsar and Aminfar 2010	Steptoe / Morex
QFmv5H	Salinity Fv/Fm	Salinity	5H	MQTL5H.7	4.77	20.64	150	132	167.8	Siahsar and Aminfar 2010	Steptoe / Morex
QRDmax.5H	Drought maximum revival rate	Drought	5H	MQTL5H.8	3.18	11.1	161	145	176.5	Zhang et al. 2005	Mona / Hordeum spontaneum
QWSC5Hb	Salinity water soluble carbohydrate	Salinity	5H	MQTL5H.7	4.75	19.15	165	146	302.7	Siahsar and Aminfar 2010	Steptoe / Morex
QSA.5H.2	Salt germination ABA	Salinity	5H	MQTL5H.8	30.4	65	169	169	169	Mano and Tekeda 1997	Harrington / TR309
QSG.5H	Salt tolerance germination	Salinity	5H	MQTL5H.8	16.4	41.1	169	169	169	Mano and Tekeda 1997	Harrington / TR307
qNAK6s	Salinity Na+ / K+ ratio	Salinity	6H	Not projected	6.1	29.81	3.98	2.6	5.36	Xue et al. 2009	CM72 / Gairdner
QTL-rp6H	Waterlogging root porosity	Waterlogging	6H	MQTL6H.1	3.69	10.4	26.55	19.4	33.67	Zhang et al. 2014	Franklin / Yerong
WL3.6	Waterlogging tolerance score (3 weeks)	Waterlogging	6H	MQTL6H.2	7.56	11.4	49	35.9	62.13	Zhou 2011	Franklin / Yerong
qSPL6s	Salinity spikes per line	Salinity	6H	MQTL6H.1	7.71	27.81	38.1	38	38.16	Xue et al. 2009	CM72 / Gairdner
Qgy-tera_6H.a	Drought grain yield	Drought	6H	MQTL6H.2	4.729	13	52	39.1	64.9	Korff et al. 2008	Tadmor / ER/Apm
Qkw-tera_6H.a	Drought kernel weight	Drought	6H	MQTL6H.2	5.249	13.6	52	39.7	64.33	Korff et al. 2008	Tadmor / ER/Apm
Qph-tera_6H.a	Drought plant	Drought	6H	MQTL6H.2	5.033	16.4	52	41.8	62.23	Korff et al.	Tadmor /

	height									2008	ER/Apm
Qph-tera_6H.b	Drought plant height	Drought	6H	MQTL6H.2	4.642	17.5	55	45.4	64.58	Korff et al. 2008	Tadmor / ER/Apm
Qgy-tera_6H.b	Drought grain yield	Drought	6H	MQTL6H.2	4.165	17.6	55	45.5	64.53	Korff et al. 2008	Tadmor / ER/Apm
QDCC.6H	Drought chlorophyll content	Drought	6H	MQTL6H.2	3.79	18.7	55	46.5	63.49	This et al. 2000	Tadmor / ER/Apm
QTL.STDW	Salinity total dry weight	Salinity	6H	MQTL6H.2	3.07	10.1	72	54.5	89.49	Nguyen et al. 2013	Steptoe / Morex
QTL.SSDW	Salinity shoot dry weight	Salinity	6H	MQTL6H.2	3.52	11.5	72	56.6	87.36	Nguyen et al. 2013	Steptoe / Morex
QDLOP6H	Drought leaf osmotic potential	Drought	6H	MQTL6H.2	3.05	24	68	60	76.01	Teulat et al. 1998	Tadmor / ER/Apm
QTL-QY1	Hypoxia quantum yield	Waterlogging	6H	MQTL6H.3	11.89	29.09	114.5	113	116	Bertholdsson et al. 2014	SLUdt1398/Mona
QTL-QY3	Hypoxia quantum yield	Waterlogging	6H	MQTL6H.3	5.14	10.74	123.5	119	128	Bertholdsson et al. 2014	SLUdt1398/Mona
QRDmin.7H	Drought minimum revival rate	Drought	7H	MQTL7H.1	3.29	11	13	0	28.64	Zhang et al. 2005	Mona / Hordeum spontaneum
QRDmax.7H.1	Drought maximum revival rate	Drought	7H	MQTL7H.1	3.23	10.6	13	0	29.23	Zhang et al. 2005	Mona / Hordeum spontaneum
GYw1.2	Waterlogging grain yield 05-06	Waterlogging	7H	MQTL7H.2	7.45	22.53	34.9	27.1	42.67	Xue et al. 2010	Franklin / Yerong
tfy2.1-2	Waterlogging leaf chlorosis 2 weeks 2005	Waterlogging	7H	MQTL7H.2	3.62	16	42.61	35	50.25	Li et al. 2008	Franklin / TX9425
QGS.7H	Salt germination speed	Salinity	7H	MQTL7H.2	5.3	15.3	45	13	77	Mano and Tekeda 1997	Harrington / TR310

HvNax3	Salinity Na+ exclusion	Salinity	7H	MQTL7H.2	9.9	51	46	41	51	Shavrukov et al.2010	Barque-73 / CPI-71284-48
QST.TxFr.7H	Salinity tolerance score	Salinity	7H	MQTL7H.2	5.4	29.2	50.25	37.6	62.85	Fan et al. 2015	TX9425/Franklin
QTL-QY2	Hypoxia quantum yield	Waterlogging	7H	MQTL7H.3	8.76	19.69	65.5	54	77	Bertholdsson et al. 2014	SLUdt1398/Mona
QGDmax.7H.1	Drought maximum germination rate	Drought	7H	MQTL7H.3	3.78	12.5	74	60.2	87.77	Zhang et al. 2005	Mona / Hordeum spontaneum
QSl.YyFr.7H	Salinity tolerance score	Salinity	7H	MQTL7H.3	10.87	15.9	75.42	65.9	84.95	Zhou et al. 2012a	Franklin / YYXT
QSlwd.YG.7H	Salinity tolerance winter	Salinity	7H	MQTL7H.3	7.09	16.1	80.4	71	89.81	Ma et al. 2015	YSM1/Gairdner
QSlww.YG.7H	Combined salinity and waterlogging tolerance winter	Combined salinity and waterlogging	7H	MQTL7H.3	6.35	13.3	82.3	70.9	93.69	Ma et al. 2015	YSM1/Gairdner
QL1L.7H	Drought first leaf length	Drought	7H	MQTL7H.4	3.01	16.8	125	113	136.8	Chen et al. 2010	WQ23-38 / MA10-30
QWl.TxNn.7H	Waterlogging tolerance score (9 weeks)	Waterlogging	7H	MQTL7H.4	7.68	12	125	113	136.7	Xu et al. 2012	TX9425 / Naso Nijo
QTW.7H	Drought time to wilt	Drought	7H	MQTL7H.4	3.1	19.5	125	115	135.1	Chen et al. 2010	WQ23-38 / MA10-30

Consensus map and QTL projection

The physical map of barley was used as the consensus map in this study (http://barleygenomeapplications.com/default_2.aspx). BioMercator V4.2 (Arcade et al. 2004) (https://urgi.versailles.inra.fr/Tools/BioMercator-V4) was used to project QTL and refine QTL positions from different populations and studies onto one consensus map. The projection of QTL on barley physical map was based on LOD scores, phenotypic variation explained by each QTL, confidence intervals and QTL positions. The positions of the 195 major QTL were based on the positions of flanking markers on the consensus map. In terms of markers without physical positions, the closest markers of the QTL flanking markers from the reference were used to project QTL on the physical map. For those QTL lacking flanking markers and confidence intervals, positions of the closest markers to these QTL were selected as the positions of QTL on the reference map. A 95% confidence interval was calculated based on the approach: confidence interval = $530 / N \times R^2$ (Darvasi and Soller 1997). Where N was the population size and R^2 was the proportion of phenotypic variance of QTL.

Meta-analysis of QTL

A meta-QTL is an integrated QTL resulting from several experiments. It is the "actual" QTL locations underlying the distribution of the observed QTL on the genome (Goffinet and Gerber 2000). Meta-analysis was conducted with BioMercator V4.2, including algorithms from the MetaQTL software (Arcade et al. 2004, Veyrieras, Goffinet and Charcosset 2007, Goffinet and Gerber 2000, Sosnowski, Charcosset and Joets 2012) (https://urgi.versailles.inra.fr/Tools/BioMercator-V4). Meta-analysis firstly determined the number of meta-QTL (MQTL) in the physical map on each chromosome from different experiments based on AIC (Akaike Information Content), AICc (AIC correction), AIC3 (AIC 3 candidate models), BIC (Bayesian information criterion) and AWE (average weight of evidence). The number was considered the best fit to carry out meta-analysis when the values of the model selection criteria were the lowest in at least three of the five models (Chardon et al. 2014). Calculated QTL from the optimum model are regarded as the meta-QTL (MQTL)

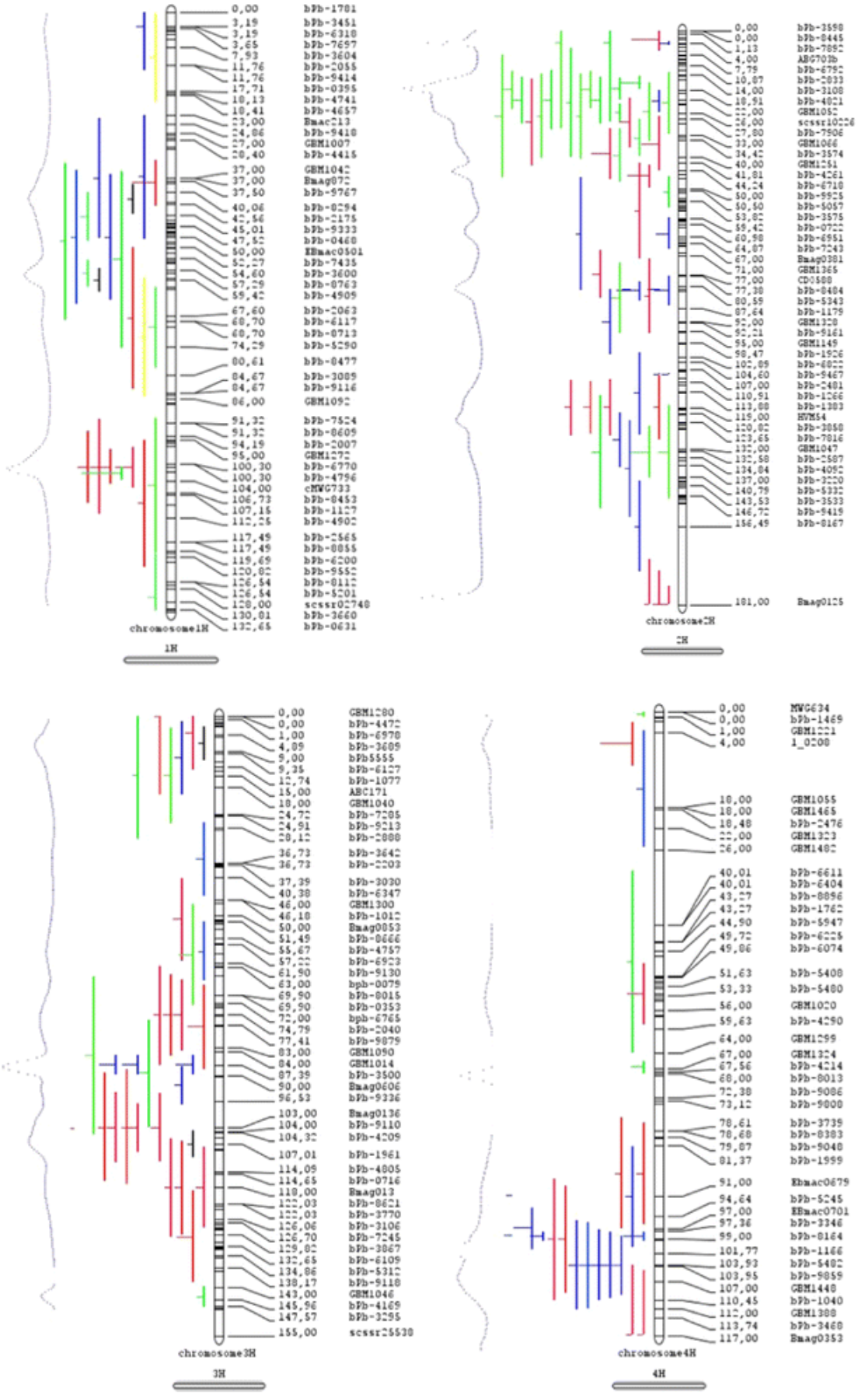
(Goffinet and Gerber 2000). The positions and 95% confidence intervals of each MQTL were calculated.

Searching for candidate genes

The confidence intervals (cM) of identified MQTL on the barley physical map were used to search for the candidate genes in barley on the website (http://barleygenomeapplications.com/default_2.aspx) with 'annotated gene' tool.

Results

The 195 major QTL for abiotic stress tolerance were projected on different chromosomes (Fig. 6.1 and Table 6.2). Chromosome 2H had the largest number of major QTL (55) and chromosome 6H had the least number of major QTL (15) for abiotic stress tolerance. There were 72 major QTL for drought tolerance, 70 major QTL for salinity tolerance, 48 major QTL for waterlogging tolerance, and 5 major QTL for combined salinity and waterlogging tolerance in barley. Each chromosome had at least 7 major QTL for drought tolerance. Most major QTL for salinity tolerance were on chromosomes 2H (23) and 5H (21). In terms of waterlogging tolerance, chromosome 2H had the most number of major QTL (15).


chromosome1H
1H
chromosome2H
2H
chromosome3H
3H
chromosome4H
4H

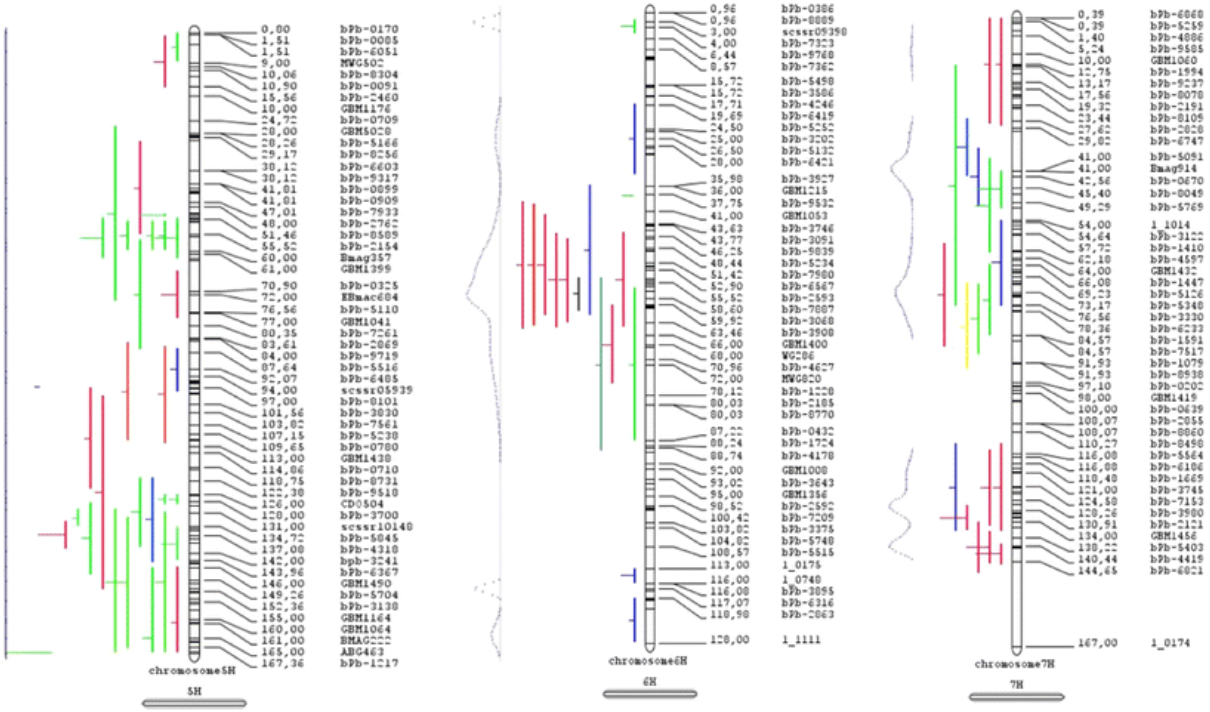


Figure 6.1 Summarized major QTL for abiotic stress tolerance [drought (*red*), salinity (*green*), waterlogging (*blue*), combined salinity and waterlogging (*yellow*), and calculated MQTL (*black*)] on the barley physical map. Common used *markers* and genetic distance (cM) are shown on the right of chromosomes. The *dotted line* on the left of chromosome is the density curve of QTL on each chromosome (Chardon et al. 2004).

Table 6.2 The number of major QTL for abiotic stress tolerance on different chromosomes.

	Drought	Salinity	Waterlogging	Combined salinity and waterlogging	Total
1H	7	8	5	2	22
2H	16	23	15	1	55
3H	16	6	7		29
4H	8	3	10		21
5H	9	21	3	1	34
6H	7	4	4		15
7H	9	5	4	1	19
Total	72	70	48	5	195

A total of 37 MQTL (~ 19%) of the initial 195 major QTL for abiotic stress tolerance were detected based on meta-analysis (Fig. 6.1 and Table 6.3). Apart from chromosome 5H, all the other chromosomes showed the peaks of density curve (Fig. 6.1), suggesting the target regions to improve abiotic stress tolerance in barley. There were 6 MQTL on chromosome 2H, with 53 initial major QTL. Each MQTL on chromosome 2H was formed with at least three initial QTL. Only three MQTL were detected on chromosome 6H. Among all the 37 identified MQTL, two MQTL were formed with QTL from six different populations and four MQTL were formed with QTL from five different populations. The QTL from different populations appeared to be unique. Meta-analysis also reduces the confidence intervals of MQTL from original 18.7 cM on average to 5.5 cM on average of each MQTL. Each MQTL had an average of 112 candidate genes based on the physical positions of MQTL (Table 6.3). MQTL6H.1 had the lowest confidence interval of 0.1 cM (38.1 cM – 38.2 cM on chromosome 6H), resulting in no candidate genes on MQTL6H.1. No candidate genes were found on MQTL2H.6 and MQTL7H.5 due to confidence intervals of less than 1.5 cM. There were more than 600 candidate genes on MQTL3H.2 and MQTL4H.2.

Table 6.3 Summary of the detected MQTL for abiotic stress tolerance

MQTL	Chromosome	Flanking markers	MQTL position	MQTL confidence interval (cM)	Number of initial QTLs	Number of studies	Number of populations	candidate genes
MQTL1H.1	1H	bPb-1781-bPb-9718	5.4	10.8	2	2	2	114
MQTL1H.2	1H	bPb-8481-GBM1451	41.7	6.7	6	3	3	70
MQTL1H.3	1H	Glb1-ABC160	59.5	5.3	6	5	3	195
MQTL1H.4	1H	ABC257	102.2	2.6	7	4	4	30
MQTL1H.5	1H	scssr02748-bPb-3201	129.8	10.4	1	1	1	112
MQTL2H.1	2H	bPb-6792	18.8	2.3	21	8	6	25
MQTL2H.2	2H	GBM1251-bPb-4875	44.8	4.7	6	4	4	54
MQTL2H.3	2H	Bmac684-Bmag0381	63.9	9.8	3	3	3	199
MQTL2H.4	2H	bPb-4377	81.9	0.3	8	4	4	11
MQTL2H.5	2H	HVM54-bPb-6688	124.2	6.5	10	4	3	62
MQTL2H.6	2H	Bmag0125	180.1	1.5	4	3	3	0
MQTL3H.1	3H	bPb-6978-bPb-5555	7.1	8.5	5	4	4	63
MQTL3H.2	3H	GBM1300-GBM1110	51.5	9.5	4	4	4	613
MQTL3H.3	3H	GBM1014-Bmag0606	87.1	3.0	10	5	5	51
MQTL3H.4	3H	Bmag0136-bPb-3630	107.1	7.0	8	2	1	125
MQTL3H.5	3H	GBM1046	144.7	1.7	2	2	2	15

MQTL4H.1	4H	MWG634-GBM1221	0.6	1.0	3	3	3	20
MQTL4H.2	4H	scssr20569-GBM1509	52.4	11.0	2	2	2	695
MQTL4H.3	4H	GBM1299-GBM1324	66.7	2.4	1	1	1	45
MQTL4H.4	4H	EBmac0701-bPb-9859	98.6	1.2	13	6	4	58
MQTL4H.5	4H	Bmag0353	117.0	5.2	2	1	1	3
MQTL5H.1	5H	scssr02306-MWG502	6.0	7.0	2	2	2	36
MQTL5H.2	5H	bPb-2762-ABC324	50.5	1.0	2	2	2	47
MQTL5H.3	5H	Bmag337-Bmag357	56.0	4.0	6	4	3	21
MQTL5H.4	5H	ABC302-GBM1041	72.0	12.2	2	2	2	137
MQTL5H.5	5H	scssr05939-bPb-8101	95.4	9.2	5	5	3	165
MQTL5H.6	5H	CDO504-bPb-3700	127.4	2.1	3	2	2	14
MQTL5H.7	5H	scssr10148-GBM1054	135.1	3.6	10	7	5	55
MQTL5H.8	5H	scssr03907	168.8	1.0	3	2	2	43
MQTL6H.1	6H	GBM1215	38.1	0.1	2	2	2	0
MQTL6H.2	6H	cdo497-bPb-3746	57.7	6.8	10	5	3	380
MQTL6H.3	6H	1_0748	115.4	0.5	2	1	1	3
MQTL7H.1	7H	bPb-6868-bPb-8660	13.2	20.4	2	1	1	206
MQTL7H.2	7H	bPb-5091-bPb-9601	43.3	7.0	5	5	4	67
MQTL7H.3	7H	bPb-2379-GBM1472	76.0	9.7	5	4	4	299
MQTL7H.4	7H	Ebmac755-GBM1456	131.3	6.1	4	2	2	122
MQTL7H.5	7H	BMAG135	142.0	1.3	3	2	2	0

Discussion

Drought tolerance in barley

Among all the abiotic stresses limiting crops yield, drought is one of the most important in agriculture and breeders have made great efforts trying to improve drought tolerance in crops (Cattivelli et al. 2008, Tuberosa and Salvi 2006). Drought is a complex quantitative trait, controlled by many genes and numerous physiological mechanisms, such as early flowering time, plant height, higher K^+ contents and osmotic adjustment (Cattivelli et al. 2008, Shabala and Pottosin 2014). Accurate phenotyping of drought tolerance remains the challenge for plant breeders to select drought tolerant genotypes (Hu and Xiong 2014, Tuberosa 2012). Different traits have been used to identify drought tolerance QTL (Table 6.1). These traits include late leaf senescence (Guo et al. 2008, Sayed et al. 2012), root system (Chen et al. 2010), osmotic adjustment (Diab et al. 2004), relative water content (Teulat et al. 2003) and yield related traits (Korff et al. 2008).

MQTL1H.4 were formed with five initial QTL for drought tolerance as shown in two studies (Korff et al. 2008, Sayed et al. 2012). The traits used as tolerence critaria include wilting score (Sayed et al. 2012), heading date, early vigour, days of maturity and days of grain filling period. All of these traits were positively correlated with yield (Korff et al. 2008). Early flowering has been regarded as an effective trait to improve drought tolerance (Blum 2005, Salvi and Tuberosa 2005), escaping drought stress during flowering stage (Tuberosa 2012). Meta-analysis of flowering traits also refined positions of QTL in maize (Chardon et al. 2004).

On MQTL1H.4, totally 30 candidate genes were identified. Putative ATP-dependent Clp protease ATP-binding subunit ClpX1 (CLPX) and Cytocrome P450 family protein were both expressed in drought susceptible rice (Rabello et al. 2008). Overexpression of *lipid transfer protein 3* enhanced drought tolerance in *Arabidopsis* (Guo et al. 2013).

MQTL3H.3 included four QTL for drought tolerance from two different studies (Fan et al. 2015, Korff et al. 2008) with three being based on agronomic traits, i.e. plant height,

peduncle length and peduncle extrusion (Korff et al. 2008). MQTL3H.3 included 51 candidate genes. Different zinc finger protein gene was found to improve drought tolerance in different plant species. Expression of CCCH-type zinc finger gene *OsTZF1* is induced by drought stress in rice (Jan et al. 2013). A C2H2-type zinc finger protein gene *GmZFP3* in soybean showed negative impact on drought tolerance in transgenic *Arabidopsis* (Zhang et al. 2016a). *IBZFP1* is encoding a C2/H2 zinc finger protein gene from sweetpotato, improving drought tolerance in transgenic *Arabidopsis* (Wang et al. 2016a). Overexpression of another C2H2-type zinc finger protein gene *GsZFP1* in transgenic *Arabidopsis* also enhanced drought tolerance (Luo et al. 2012).

MQTL3H.4 was formed with eight drought tolerant QTL from two populations based on different physiological traits, wilting score, peduncle length, water soluble carbohydrate contents, and plant height (Sayed et al. 2012, Diab et al. 2004, Korff et al. 2008). MQTL6H.2 was formed with seven QTL for drought tolerance based on grain yield, kernel weight, and plant height (Korff et al. 2008) and chlorophyll content (This et al. 2000). MQTL3H.4 and MQTL6H.2 had relatively large confidence intervals (7.0 cM and 6.8 cM), resulting in the large amount of condidate genes (more than 100).

Salinity tolerance in barley

Salinity tolerance is also a complex trait, controlled by many minor QTL (Flowers and Flowers 2005). Slow progress was made to improve salinity tolerance with MAS in crops during the past few years although many QTL for salinity tolerance were identified (Ashraf and Foolad 2013). There are two phases of growth reactions in crops to salinity stress (Munns and Tester 2008). The first phase is the osmotic effect to crops, reducing water uptake by crops, that is similar to drought effects. The second phase is the ion toxicity caused by Na^+ and/or Cl^- that inhibit crop growth.

Many physiological traits are regarded as salinity tolerant mechanisms (Colmer et al. 2005, Munns 2005). This includes: osmotic adjustment; Na^+ exclusion from uptake; control of xylem ion loading; efficient vacuolar Na^+ sequestration; reactive oxygen species (ROS) detoxification; and cytosolic K^+ homeostasis (Flowers and Colmer 2008, Munns and Tester 2008).

The fine mapped QTL for salinity tolerance were on chromosome 1H and 7H. *HvNax4* is the locus lowering the shoot Na^+ contents in barley on MQTL1H.4 (Rivandi et al. 2011). This locus was fine mapped and 34 candidate genes were identified (Rivandi et al. 2011). Possibly, the detected QTL for salinity tolerance at seedling stage on MQTL1H.4 had the same genes with *HvNax4* (Mano and Takeda 1997). MQTL1H.4 was also the hot spot to improve drought tolerance, including five drought tolerant QTL (discussed above), showing the possibility of improving drought tolerance and salinity tolerance simultaneously.

Among the identified 30 candidate genes on MQTL1H.4, over expression of heavy metal transport/detoxification superfamily protein was detected in transgenic *Arabidopsis* under salinity conditions (Yokotani et al. 2013). Based on meta-analysis in rice, the pentatricopeptide repeat (PPR)-containing protein-like gene was identified as the candidate gene for improving rice yield on different chromosomes (Swamy et al. 2011).

MQTL7H.2 formed a fine mapped major locus for salinity tolerance *HvNax3*, explaining 51% phenotypic variance with a LOD value of 9.9 (Shavrukov et al. 2010). Neither *HvNax3* nor *HvNax4* were able to influence K^+ contents in barley (Rivandi et al. 2011, Shavrukov et al. 2010), while *HvNax3* was shown to lower the sodium accumulation in leaves. The physiological mechanisms of this reduction remains a matter of conjecture. Several candidate genes were identified in the *HvNax3* locus with colinearity in rice and *Brachypodium*. From meta-analysis, 67 candidate genes were also identified in the locus *HvNax3*. Which of these candidate genes play a role in controlling Na^+ content in the shoot remain to be investigated in future experiments. It was suggested earlier that other Nax loci, *Nax 1* and *Nax 2*, enhance the retrieval of Na^+ back into the root stele via HKT1;4 or HKT1;5 (Munns et al. 2012). However, more recent studies have shown that Nax loci also reduces the rate of Na^+ loading into the xylem via SOS1 Na^+/H^+ exchanger in wheat (Zhu et al. 2016). It remains to be determined which of these mechanisms is conferred by *Nax3* loci. QTL for germination speed under salinity stress (Mano and Takeda 1997) and salinity tolerance score (Fan et al. 2015) were also located in MQTL7H.2. It is probable that these two QTL are also controlled by the locus *HvNax3*.

The *TaMyb1* gene was suggested to be involved in the signalling pathways of waterlogging and salinity stresses (Lee et al. 2007). Overexpression of another Myb transcription factor gene, *JAmyb*, contributed to salinity tolerance by stimulating abiotic stress tolerant genes,

such as osmotic adjustment and ROS scavenging, in rice and *Arabidopsis* (Yokotani et al. 2013). Another Myb transcription factor, SRM1, is able to regulate the ABA biosynthesis and signalling related genes in *Arabidopsis* under salinity stress (Wang et al. 2015). A calmodulin-like protein OsMSR2 identified in rice was found to improve drought and salinity tolerance by regulating stress related genes in ABA-mediated pathways. Expression of OsMSR2 showed improved drought and salinity tolerance in *Arabidopsis* (Xu et al. 2011).

Generally, root K^+ retention ability is strongly associated with salinity tolerance in barley (Chen et al. 2005, Chen et al. 2007). One QTL for root K^+ under salinity stress was identified on chromosome 1H, located on MQTL1H.3 (Nguyen et al. 2013). MQTL1H.3 was formed with four initial QTL for salinity tolerance from three different experiments from the same mapping population (Steptoe / Morex). Different traits were used among these three initial QTL: leaf injury, root K^+, and chlorophyll content. All of these three salinity tolerant QTL on MQTL1H.3 were from the seedling growth stages.

MQTL2H.1 was formed with 21 major QTL, including two for waterlogging tolerance, one for combined salinity and waterlogging tolerance, three for drought tolerance, and 15 for salinity tolerance. This region is the main area contributing to salinity tolerance in barley, at both seedling and vegetative growth stages. MQTL2H.1 included QTL for salinity tolerance based on leaf yellowing, number of yellow leaves, leaf dry matter, and proline, Na^+, K^+, and Cl^- contents in leaves.

There were 25 candidate genes on MQTL2H.1, including two candidate genes of particular interest. In soybean seedlings, the protein flavonol 4′-sulfotransferase was downregulated when placed under combined salinity and waterlogging conditions (Alam et al. 2011). The cytochrome P450-like gene was upregulated in waterlogged rape seedlings (Lee et al. 2014). These two candidate genes on MQTL2H.1 can be further explored to improve abiotic stress tolerance in barley.

MQTL5H.3 was formed with six QTL for salinity tolerance from three different studies. Glutamate receptor was the candidate gene on MQTL5H.3. Glutamate receptor is one of the factors inducing K^+ efflux under abiotic stresses (Demidchik et al. 2014). Maintaining high cytosolic K^+ level with lower K^+ efflux is crucial for abiotic stress tolerance in barley (Shabala and Pottosin 2014), and a causal link exists between cytosolic K^+ concentration and

the ability of a cell to undergo programmed cell death (e.g. senescence) (Demidchik et al. 2014, Shabala et al. 2010).

Waterlogging tolerance in barley

The factor that has impeded the progress of improving waterlogging tolerance in barley is the low heritability of plant yield under waterlogging conditions (Collaku and Harrison 2005, Zhou 2010). Despite the advanced genotyping technology, accurate phenotyping remains to be a challenge in plant breeding for waterlogging tolerance (Zhou 2011). Agronomic traits were widely used to screen waterlogging tolerance in barley, rice and maize (Qiu et al. 2007, Xu and Mackill 1996, Zhou 2010). Visual symptom of leaf yellowing is the main indicator of waterlogging tolerance in barley breeding programs (Table 6.1). Utilizing physiological traits associated with waterlogging tolerance, such as higher K^+ contents, is required in waterlogging breeding programs (Shabala 2011, Shabala et al. 2014). Even in breeding, only a few physiological traits have been utilized and none of the genes encoding these traits have been cloned (Collins, Tardieu and Tuberosa 2008). More convenient and reliable physiological traits should be further explored to screen waterlogging tolerance.

Aerenchyma formation in roots is a reliable and faster method to detect waterlogging tolerance, compared with leaf chlorosis (Zhang et al. 2015b, Zhang et al. 2016d). Root porosity is the percentage of gas volume per root volume, widely used as an indicator of aerenchyma formation (Colmer 2003a). Aerenchyma provides an internal system of gas-filled spaces to improve oxygen supply in waterlogged roots (Evans 2004). MQTL4H.4 was formed with nine QTL for waterlogging tolerance, including one fine mapped QTL for aerenchyma formation and two QTL for root porosity under waterlogging conditions (Zhang et al. 2016d). The seven QTL were from the population of Yerong/Franklin and two QTL from YYXT/Franklin (Broughton et al. 2015, Li et al. 2008, Zhou 2011). MQTL4H.4 was positioned at 98.6 cM with confidence interval of 1.2. MQTL4H.4 and can be used in MAS in breeding to improve waterlogging tolerance in barley.

There were 58 candidate genes on MQTL4H.4 contributing to waterlogging tolerance in barley. Members of the family of NAC domain proteins were increased during leaf senescence in *Arabidopsis* (Buchanan-Wollaston et al. 2005). In addition, the NAC domain-containing gene ANAC102 was induced as an important regulator of seed germination under

waterlogging conditions (Christianson et al. 2009). In waterlogging tolerant maize, calcium-dependent lipid-binding (CaLB domain) protein showed increasing abundance (Yu et al. 2015). Catalase was one of the antioxidant enzymes reducing the oxidative stress under waterlogging conditions (Zhang et al. 2015b). Cytochrome P450-like gene and glutathione-S-transferase on MQTL4H.4 were upregulated in waterlogged rape seedlings (Lee et al. 2014). However, glutathione-S-transferase gene was downregulated in waterlogged cucumber (Qi et al. 2012). Glycosyltransferase genes, which are involved in cytokinin inactivation, showed decreased expression under waterlogging conditions (Christianson et al. 2010, Qi et al. 2012). *LOB-DOMAINCONTAINING PROTEIN 41* (*LBD41*) is likely a repressing factor in submerged *Arabidopsis* (Voesenek et al. 2016). The mitochondrial serine acetyltransferase gene was upregulated in waterlogged rape seedlings (Christianson et al. 2010).

Lysigenous aerenchyma formation candidate genes have been identified in maize (Rajhi et al. 2011). The identified candidate genes, NAC domain transcription factor gene and glutathione-S-transferase gene, were both located to MQTL4H.4 (Rajhi et al. 2011). MQTL4H.4 also included one fine mapped QTL for aerenchyma formation under waterlogging conditions in barley (Zhang et al. 2016d). Further studies are needed to identify genes controlling aerenchyma formation under waterlogging conditions in barley.

Based on chlorophyll fluorescence, there were two major QTL under hypoxia conditions identified on chromosome 6H, explaining 39.8% of the phenotypic variance (Bertholdsson et al. 2015). This suggests that QTL can also be fine mapped and used for MAS. Meta-analysis projected these two QTL on MQTL6H.4 on the physical map.

MQTL7H.2 also included two QTL for waterlogging tolerance. Ethylene response factors gene is also located in the region of MQTL7H.2 and can be the candidate gene for waterlogging tolerant QTL on MQTL7H.2 (Xu et al. 2006). Increased transcripts of a Myb transcription factor *TaMyb1* gene was identified in wheat under waterlogging conditions and combined salinity and waterlogging stress (Lee et al. 2007).

Combined drought and salinity stresses

Plants are usually subjected to combined drought and salinity in both natural and agricultural systems (Roy, Tucker and Tester 2011). The direct effect from drought and salinity stresses is the reduction of photosynthesis and cell growth (Chaves, Flexas and Pinheiro 2009). Osmotic

adjustment is one of the crucial mechanisms of drought tolerance in crops, enhancing photosynthetic rates through water uptake and cell turgor (Cattivelli et al. 2008). Osmotic adjustment is also the key trait for salinity tolerance in the first phase of salinity stress (Munns and Tester 2008). MQTL2H.4, MQTL5H.1 and MQTL6H.2 were all formed with one QTL for leaf osmotic potential under drought stress (Teulat, Borries and This 2001, Teulat et al. 1998) and QTL for salinity tolerance. These findings suggested the possibility of using osmotic adjustment to improve drought and salinity tolerance simultaneously, as well as improving combined drought and salinity tolerance.

MQTL1H.4 was formed with five QTL for drought tolerance and two QTL for salinity tolerance. One QTL for salinity tolerance, *HvNax4*, which lowers the shoot Na^+ content in barley, is mapped to a 200 kb interval within this region (Rivandi et al. 2011). MQTL1H.4 can be a possible region controlling combined drought and salinity tolerance in barley. Until now experiments regarding plant response to combined drought and salinity stresses are limited (Ahmed et al. 2013). QTL for combined drought and salinity tolerance have not been identified yet.

Drought would aggravate the ion toxicity caused by Na^+ and Cl^-, thereby impeding plant growth (Ahmed et al. 2013). Under combined drought and salinity stresses, relatively more tolerant wild barley genotypes were shown to have higher K^+ contents and K^+/Na^+ ratio than the relatively intolerant genotypes (Ahmed et al. 2013). MQTL2H.1 was formed with 16 QTL for salinity tolerance and three QTL for drought tolerance. One QTL for salinity tolerance was based on shoot K^+ contents. MQTL3H.1 was formed with two drought tolerant QTL from two different populations (Diab et al. 2004, Zhang et al. 2005), and two salinity tolerant QTL based on the plant Na^+/K^+ ratio and shoot Na^+/K^+ ratio (Nguyen et al. 2013). MQTL2H.1 and QTL3H.1 both illustrated that maintaining higher K^+ contents helped plants to adapt better to the drought, salinity, and combined drought and salinity stresses.

Combined salinity and waterlogging stresses

Waterlogged soils can be also affected by salinity. Under combined salinity and waterlogging stresses, severe damage occurs in barley (Colmer et al. 2005). Oxygen deprivation in waterlogged soils inhibited the ATP production in plants (Bailey-Serres and Voesenek 2008). Reduced ATP in plants leads to increased Na^+ and decreased K^+ levels in leaves under

combined stress (Barrett-Lennard and Shabala 2013, Zeng et al. 2013). Aerenchyma provides an internal system of gas-filled spaces to improve oxygen supply to waterlogged roots, leading to increased energy in plants (Evans 2004). Therefore, it is proposed that aerenchyma formation can be an effective mechanism in plants under combined salinity and waterlogging stresses (Colmer and Flowers 2008). Maintaining lower Na^+ and higher K^+ content in leaves is another key mechanism for improving combined salinity and waterlogging tolerance (Zeng et al. 2013). So far, there is only one experimental study detecting QTL for combined salinity and waterlogging tolerance in barley (Ma et al. 2015).

In our study, MQTL4H.4 was formed with nine QTL for waterlogging tolerance, including one fine mapped QTL for aerenchyma formation and two QTL for root porosity under waterlogging conditions (Zhang et al. 2016d). However, no salinity tolerant QTL were located on MQTL4H.4. Another QTL for root porosity under waterlogging conditions was on MQTL6H.2, and without any QTL for salinity tolerance. In addition, MQTL1H.4 with fine mapped salinity tolerant QTL *HvNax4*, was not formed with QTL for waterlogging tolerance. MQTL7H.2 was formed with fine mapped salinity tolerant QTL *HvNax3* and two QTL for waterlogging tolerance.

QTL for combined salinity and waterlogging tolerance were projected on MQTL1H.1 MQTL1H.3, MQTL2H.1, MQTL5H.6, and MQTL 7H.3 (Ma et al. 2015). MQTL2H.1 and MQTL7H.3 both included QTL for salinity tolerance and waterlogging tolerance. MQTL2H.1 and MQTL7H.3 indicated the possibility to improve combined salinity and waterlogging tolerance.

Importance of marker validation and limitations of meta-analysis

A major objective of QTL studies is to find QTL that can be implemented into breeding programs via MAS. The major objective of barley breeding is high yield, combined with greater malting quality and insensitivity to biotic and abiotic stresses. QTL has been successful for introgressing and pyramiding major effect genes. However, there are still many traits of interest facing great challenges since traits are controlled by many QTL with small effects.

A meta-analysis of QTL associated with abiotic stresses has been performed in barley (Li et al. 2013). Overall 35 experiments under both control and stress conditions, with 337 major or

minor QTL on drought, salinity, waterlogging, low temperature, mineral toxicity or deficiency being included in their study (Li et al. 2013). In our study, a larger number and the latest QTL (632 overall) for drought, salinity, and waterlogging tolerance were investigated. Before meta-analysis, we excluded the parameters under control conditions and the QTL with minor effects. The QTL controlling the yield related traits under stresses might be the QTL for yield related traits, rather than the stress tolerant QTL (Jones 2007). The change of traits under stress conditions should be compared with the traits under control conditions. We only used major QTL for stress tolerance in barley to perform meta-analysis since MAS was successful in crop breeding with one or two major genes controlling stress tolerance. To our knowledge, this is the first meta-analysis that projected all the QTL on the barley physical map, with previous meta-analysis generating the consensus map from the markers common to the different population maps (Khowaja et al. 2009, Li et al. 2013, Zhang et al. 2010). A limited number of common markers from different populations resulted in the inaccurate QTL positions on the consensus map. In our study, we used the position of the QTL flanking markers on the barley physical map to refine the positions of abiotic stresses tolerant QTL from different studies. The positions of MQTL on the barley physical map were also used to search the candidate genes. Identified candidate genes on the physical map provide meaningful information for further MAS and positional clone.

Meta-analysis is able to integrate the different QTL from different populations into one consensus map. Meta-analysis has also successfully validated the major QTL for abiotic stress tolerance in barley reducing the confidence interval of MQTL. After primary QTL mapping, the mapped QTL was located within a chromosome region so that the confidence interval was up to 50 cM (Table 6.1). Chromosome regions within 10 cM include several hundred of genes (de Dorlodot et al. 2007, Salvi and Tuberosa 2005). Fine mapping is widely used to refine the QTL less than 1 cM between flanking markers to search for candidate genes and positional cloning of QTL for abiotic stress tolerance (de Dorlodot et al. 2007, Rivandi et al. 2011, Semagn et al. 2013, Shavrukov et al. 2010). QTL cloning has enhanced the exploitation of functions of tolerant genes and the allelic variation in germplasm (Ashraf and Foolad 2013). Meta-analysis provided another method to refine the locations of QTL by lowering the confidence interval (de Dorlodot et al. 2007). The calculated meta-QTL provides breeders with target regions on consensus map for further MAS. However, how effective and accurate is the reduction of confidence interval for searching candidate genes by

meta-analysis remains unknown unless that the number of observed QTL is more than five (Veyrieras et al. 2007). In addition, recombination might break the linkage between markers and target QTL. Further experiments are therefore required to explore the MQTL from meta-analysis before it can become an effective tool in crop breeding.

Conclusions

Both MQTL3H.4 and MQTL6H.2 were target regions controlling drought tolerance in barley. Further experiments are required to fine map these regions for the effective use of MAS in drought tolerance breeding in barley. Fine mapped QTL for salinity tolerance, *HvNax4* and *HvNax3*, were validated on MQTL1H.4 and MQTL7H.2, respectively. MQTL1H.4 was formed with fine mapped salinity tolerant *HvNax4* and 5 initial major QTL for drought tolerance. MQTL1H.4 provides breeders with the possibility of improving drought tolerance and salinity tolerance simultaneously and thereby improving barley performance under combined drought and salinity stresses. MQTL7H.2 was formed with a fine mapped major locus for salinity tolerance, *HvNax3*, two other QTL for salinity tolerance and two QTL for waterlogging tolerance. Genes for ethylene response factors and Myb transcription factor are possible candidate genes for salinity tolerant locus *HvNax3*. Improved salinity tolerance, waterlogging tolerance, and combined salinity and waterlogging tolerance can be achieved by selecting MQTL7H.2. MQTL 2H.1 and MQTL5H.3 were also target regions improving salinity tolerance. MQTL4H.4 is the main region controlling waterlogging tolerance in barley, including fine mapped QTL for aerenchyma formation under waterlogging conditions. The genes for NAC domain transcription factor and glutathione-S-transferase are candidate genes for aerenchyma formation under waterlogging conditions in barley. Identified MQTL and candidate genes provide breeders with target regions to improve drought, salinity, and waterlogging tolerance in barley.

Chapter 7: General conclusions and discussion

Conclusion

1 Knowledge on mechanisms associated with waterlogging tolerance in different species, such as the aerenchyma formation and radial oxygen loss was summarized. The utilization of marker assisted selection in plant breeding was also reviewed and indicated the future research areas.

2 Faster aerenchyma formation is associated with waterlogging tolerance in barley. By contrast, antioxidants activities and metabolites like GABA and lactic acid in leaves are not associated with waterlogging tolerance in barley.

3 A reliable and fast method to detect waterlogging tolerance was developed based on aerenchyma formation after 7 days waterlogging treatment. One QTL on chromosome 4H explaining 42% phenotypic variance was identified in Franklin/Yerong DH population based on aerenchyma formation after 7 days waterlogging treatment.

4 A new DH population from the cross between Franklin and wild barley TAM407227 was developed. A new major allele for aerenchyma formation on chromosome 4H was detected. This QTL explained 76.8% phenotypic variance with an LOD value of 51.4. The high density linkage map can be further used in marker assisted selection to improve waterlogging tolerance in barley.

5 Overall 195 major QTL for drought, salinity and waterlogging tolerance in barley were summarized. Meta-analysis was used to refine these major QTL positions on barley physical map and the candidate genes at each MQTL were identified. All of the identified MQTL provided breeders valuable resources to improve barley abiotic stresses tolerance sulmuntaneously.

Discussion

Waterlogging is one of the important limiting conditions of plant yield and productivity. Every year, yield loss of barley due to waterlogging ranges from 20-25% (Setter et al. 1999). Waterlogging causes many adverse effects on plant growth, including physiological and biochemical problems. The gas diffusion under waterlogging conditions is 10,000-fold slower in solution than in air (Armstrong 1979). The depletion of O_2 is a major feature of flooded sites, which creates hypoxia or anoxia around plant tissues. This leads to acute energy crises and very significant alterations in cell metabolism (and associated yield penalties) (Colmer and Voesenek 2009).

There are several possible options plants can use to adjust to this energy crisis. Maintaining adequate oxygen supply by a series of anatomical and morphological alterations in the root is one of the mechanisms to adjust to the energy crisis (Voesenek et al. 2016). One of these alterations is the formation of aerenchyma. Species with higher root porosity are more tolerant to soil flooding, and in many wetland plants, aerenchyma is well developed even in drained conditions (and can be further enhanced in waterlogged conditions), while dry land species often do not form aerenchyma at all (Colmer 2003a). Aerenchyma in roots is a special tissue with gas spaces, forming an internal system to improve the diffusion and concentration of oxygen (Colmer 2003b). The increased concentration of oxygen leads to higher respiration rates and increased energy (ATP) in roots (Drew et al. 1985, Suralta and Yamauchi 2008).

In barley, waterlogging-tolerant genotypes showed not only significantly higher adventitious root porosity than susceptible genotypes but, more importantly, a faster increase of root porosity resulting from faster development of aerenchyma. However, the changes in antioxidant enzyme activities in leaves, GABA and lactic acid contents in roots under waterlogging conditions do not appear to be targets when considering selection criteria for waterlogging tolerance in barley.

Based on these severe problems caused by waterlogging, different methods can be used to improve crop yields under waterlogging conditions. The development of waterlogging tolerant varieties is one of the most effective and economical approaches to improve production. Waterlogging conditions are variable and complex and plant tolerance to waterlogging is also a complex trait, which is controlled by many genes, some with small

effects. Therefore, progress to breed waterlogging tolerant varieties is quite slow. One of the reasons for slow progress is the unreliable screening methods. With the development of advanced genotyping methods, accurate phenotyping remains the crucial requirement to enhance marker assisted selection in breeding. Grain yield under waterlogging conditions exhibit low heritability (0.25) (Collaku and Harrison 2005). Therefore, yield under waterlogging conditions cannot be used as the direct selection criterion to improve waterlogging tolerance.

Earlier experiments have shown that root porosity in potting mixture under waterlogging stress was significantly correlated with waterlogging tolerance (Zhang et al. 2015b). However, the measurement of root porosity is time consuming and labor intensive. In this study, a faster and more accurate phenotyping method was developed to select waterlogging tolerance in barley. Aerenchyma formation after 7 days of waterlogging in commercial potting mixture can be a reliable, fast, and widely utilized approach for the selection of waterlogging tolerant barley genotypes.

This method is further used to identify QTL for aerenchyma formation under waterlogging conditions in different experiments. QTL for aerenchyma under waterlogging conditions was identified in a doubled haploid population of barley from the cross between Yerong (tolerant) and Franklin (sensitive) genotypes. The QTL for aerenchyma formation and root porosity were at the same location as that for waterlogging tolerance. Fine mapping is widely used to refine QTL positions for successful MAS and searching for candidate genes (de Dorlodot et al. 2007, Semagn et al. 2013). Seven polymorphic InDel markers were developed to fine map the major QTL for aerenchyma formation under waterlogging conditions on chromosome 4H. One major QTL for aerenchyma formation after 7 days of waterlogging treatment explained 44.0 % of the phenotypic variance. This successful QTL for aerenchyma formation can be effectively used in MAS to improve waterlogging tolerance in barley.

The wild barley TAM407227 showed significantly higher potential for enhancing waterlogging tolerance in barley (Zhang et al. 2015b). Compared with waterlogging tolerant cultivated barley Yerong, TAM407227 performed much better tolerance to waterlogging with a greater proportion of aerenchyma formation under waterlogging conditions. A new allele for aerenchyma formation was identified from a doubled haploid population of barley from the cross between TAM407227 (tolerant) and Franklin (sensitive) on chromosome 4H. The

QTL explained 76.8% of phenotypic variance in aerenchyma formation with a LOD value of 51.4.

The region on chromosome 4H controlling aerenchyma formation identified from the population of Franklin/TAM407227 is at the same position as that from both Yerong/Franklin and YYXT/Franklin populations (Zhou 2011, Zhou et al. 2012). The allele originating from wild barley TAM407227 not only exhibited a higher percentage of phenotypic variation (76.8% in TAM407227 compared to 44% in Yerong (Zhang et al. 2016c) and 39% in YYXT (Broughton et al. 2015)), but also made a much greater contribution to waterlogging tolerance than the allele from cultivated barley varieties. Of the total percentage of phenotypic variation determined by three significant QTL (46.2%), the allele on 4H contributed 34.6% (75% of total contribution). In contrast, the allele from Yerong contributed 23.9% to the overall waterlogging tolerance and 42% of all the contributions by four QTL (Zhou 2011). The allele from YYXT contributed only 5.2% to the overall waterlogging tolerance, which is only 11% of all the contributions by the four QTL (Zhou et al. 2012). Together this further confirms that aerenchyma formation is one of the most effective mechanisms for waterlogging tolerance (Armstrong 1979). These mechanisms play more important roles in waterlogging tolerance in cultivated barley, while in wild barley TAM407227 the allele controlling aerenchyma formation was shown to be most effective in improving waterlogging tolerance; thus, it can be effectively used in future breeding programs. A high resolution map of chromosome 4H in barley was constructed and provided enough markers for further MAS to improve waterlogging tolerance in barley.

Similar results have been reported in other crops. Wild relatives of maize are able to form aerenchyma without waterlogging stress (Mano et al. 2006). Wild relatives of wheat showed higher root porosity and lower radial oxygen loss under waterlogging conditions (Malik et al. 2009). These favourable traits of waterlogging tolerance in wild relatives of maize and wheat have been successfully transferred to cultivated maize and wheat (Malik et al. 2011, Mano and Omori 2013). This further suggested transferring the waterlogging tolerant allele from wild barley to cultivated barley to improve waterlogging tolerance in barley.

Drought, salinity and waterlogging are three major abiotic stresses limiting barley yield worldwide. Breeding for abiotic stress-tolerant crops has drawn increased attention, and a

large number of QTL for drought, salinity, and waterlogging tolerance in barley have been detected. However, very few QTL have been successfully used in MAS in breeding.

Overall 632 QTL for drought, salinity and waterlogging tolerance in barley were summarized. Among all these QTL, only 195 major QTL were used to conduct meta-analysis to refine QTL positions for MAS. Meta-analysis was used to map the summarized major QTL for drought, salinity, and waterlogging tolerance from different mapping populations on the barley physical map. The positions of identified meta-QTL (MQTL) on the barley physical map were used to search for candidate genes for drought, salinity, and waterlogging tolerance in barley.

Both MQTL3H.4 and MQTL6H.2 were target regions controlling drought tolerance in barley. Further experiments are required to fine-map these regions for the effective use of MAS in drought tolerance breeding in barley. Fine-mapped QTL for salinity tolerance, HvNax4 and HvNax3, were validated on MQTL1H.4 and MQTL7H.2, respectively. MQTL1H.4 was formed with fine-mapped salinity-tolerant HvNax4 and five initial major QTL for drought tolerance. MQTL1H.4 provides breeders with the possibility of improving drought tolerance and salinity tolerance simultaneously and thereby improving barley performance under combined drought and salinity stresses. MQTL7H.2 was formed with a fine-mapped major locus for salinity tolerance, HvNax3, two other QTL for salinity tolerance and two QTL for waterlogging tolerance. MQTL4H.4 was formed with nine QTL for waterlogging tolerance, including one fine-mapped QTL for aerenchyma formation and two QTL for root porosity under waterlogging conditions. MQTL4H.4 was positioned at 98.6 cM with confidence interval of 1.2. Identified MQTL and candidate genes provide breeders with target regions to improve drought, salinity, and waterlogging tolerance in barley.

Overall, waterlogging tolerant barley genotypes showed the faster development of aerenchyma under waterlogging conditions. Aerenchyma formation after 7 days of waterlogging treatment is a fast and reliable method to screen waterlogging tolerance. This screening method was further used to identify a major QTL for aerenchyma formation under waterlogging conditions in barley. This QTL was fine mapped and many molecular markers were developed. In the future, the major waterlogging tolerant allele(s) from wild barley will be transferred to cultivated barley with MAS. Developed molecular and physiological

markers for aerenchyma formation in barley will be further used in practice to improve waterlogging tolerance in barley.

Future research directions

Aerenchyma formation under waterlogging stress is one of the most effective mechanisms to provide adequate oxygen supply. In barley, the fast and reliable screening method for waterlogging tolerance based on aerenchyma formation was identified. In addition, a new allele for aerenchyma formation was identified from a wild barley accession TAM407227 on chromosome 4H. Compared to that identified in cultivated barley, this allele not only produced a greater proportion of aerenchyma but made a greater contribution to the overall waterlogging tolerance. Markers co-segregating with the trait were identified and can be effectively used in MAS. Further studies will be required to transfer this major allele for aerenchyma formation to commercial barley varieties. The developed waterlogging tolerant varieties will significantly improve grain yield to feed the world. In addition, this gene for aerenchyma formation in barley will be cloned and investigated to determine its mode of action. .

The successfully utilized submergence tolerant gene *Sub1* can be an example of using MAS to improve waterlogging tolerance in cereals through breeding. *Sub1* is the major locus for submergence tolerance on chromosome 9 in rice, contributing to 69% phenotypic variance (Xu and Mackill 1996, Xu et al. 2006, Xu et al. 2000). With MAS, *Sub1* has been successfully introgressed into different commercial varieties, greatly improving grain yield in submergence soils with no negative influences under normal conditions (Septiningsih et al. 2009, Singh et al. 2009).

Numerous lines of evidence suggest that ethylene accumulation induced by hypoxia is essential to trigger formation of lysigenous aerenchyma in waterlogged roots (Colmer et al. 2006). When the root was treated with the inhibitors of ethylene biosynthesis, aerenchyma did not develop and programmed cell death was inhibited (Rajhi et al. 2011, Yamauchi et al. 2014b). The ethylene dependent pathway for aerenchyma formation in adventitious roots is regulated differently in rice and maize (Yamauchi et al. 2016) and the ethylene independent pathway for aerenchyma formation identified in wetland species (Visser and Bögemann

2006). Further studies should be performed to identify the signalling pathway for aerenchyma formation in barley under waterlogging conditions.

References

Abiko, T., L. Kotula, K. Shiono, A. I. Malik, T. D. Colmer & M. Nakazono (2012a) Enhanced formation of aerenchyma and induction of a barrier to radial oxygen loss in adventitious roots of *Zea nicaraguensis* contribute to its waterlogging tolerance as compared with maize (*Zea mays* ssp. *mays*). *Plant Cell and Environment,* 35**,** 1618-30.

Abiko, T., M. Obara, F. Abe, K. Kawaguchi, A. Oyanagi, T. Yamauchi & M. Nakazono (2012b) Screening of candidate genes associated with constitutive aerenchyma formation in adventitious roots of the teosinte *Zea nicaraguensis*. *Plant Root,* 6**,** 19-27.

Aebi, H. & L. Packer. 1984. Methods in Enzymology. 105Academic Press, New York.

Agarwal, S. & A. Grover (2006) Molecular biology, biotechnology and genomics of flooding-associated low O2 stress response in plants. *Critical Reviews in Plant Sciences,* 25**,** 1-21.

Ahmed, I. M., F. Cao, M. Zhang, X. Chen, G. Zhang & F. Wu (2013) Difference in yield and physiological features in response to drought and salinity combined stress during anthesis in Tibetan wild and cultivated barleys. *PLoS ONE,* 8**,** e77869.

Alam, I., S. Sharmin, K.-H. Kim, Y.-G. Kim, J. Lee, J. Bahk & B.-H. Lee (2011) Comparative proteomic approach to identify proteins involved in flooding combined with salinity stress in soybean. *Plant and Soil,* 346**,** 45-62.

Alscher, R. G., N. Erturk & L. S. Heath (2002) Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *Journal of Experimental Botany,* 53**,** 1331-1341.

Anschütz, U., D. Becker & S. Shabala (2014) Going beyond nutrition: Regulation of potassium homoeostasis as a common denominator of plant adaptive responses to environment. *Journal of Plant Physiology,* 171**,** 670-687.

Apel, K. & H. Hirt (2004) REACTIVE OXYGEN SPECIES: Metabolism, Oxidative Stress, and Signal Transduction. *Annual Review of Plant Biology,* 55**,** 373-399.

Arbona, V., Z. Hossain, M. F. López-Climent, R. M. Pérez-Clemente & A. Gómez-Cadenas (2008) Antioxidant enzymatic activity is linked to waterlogging stress tolerance in citrus. *Physiologia Plantarum,* 132**,** 452-466.

Arcade, A., A. Labourdette, M. Falque, B. Mangin, F. Chardon, A. Charcosset & J. Joets (2004) BioMercator: integrating genetic maps and QTL towards discovery of candidate genes. *Bioinformatics,* 20**,** 2324-2326.

Armstrong, J. & W. Armstrong (1999) *Phragmites* die-back: toxic effects of propionic, butyric and caproic acids in relation to pH. *New Phytologist,* 142**,** 201-217.

Armstrong, J. & W. Armstrong (2001) Rice and *Phragmites*: effects of organic acids on growth, root permeability, and radial oxygen loss to the rhizosphere. *American Journal of Botany,* 88**,** 1359-1370.

--- (2005) Rice: sulfide-induced barriers to root radial oxygen loss, Fe^{2+} and water uptake, and lateral root emergence. *Annals of Botany,* 96**,** 625-638.

Armstrong, W. 1979. *Aeration in higher plants*. London: Advances in Botanical Research.

Ashraf, M. & M. R. Foolad (2013) Crop breeding for salt tolerance in the era of molecular markers and marker-assisted selection. *Plant Breeding,* 132**,** 10-20.

Axelsen, K. B. & M. G. Palmgren (2001) Inventory of the superfamily of P-type ion pumps in *Arabidopsis*. *Plant physiology,* 126**,** 696-706.

Baba, I., K. Inada & K. Tajima (1964) Mineral nutrition and the occurrence of physiological diseases. *The mineral nutrition of the rice plant***,** 173-195.

Bai, Q., M. Chai, Z. Gu, X. Cao, Y. Li & K. Liu (2009) Effects of components in culture medium on glutamate decarboxylase activity and γ-aminobutyric acid accumulation in foxtail millet (*Setaria italica* L.) during germination. *Food Chemistry,* 116**,** 152-157.

Bailey-Serres, J. & R. Chang (2005) Sensing and signalling in response to oxygen deprivation in plants and other organisms. *Annals of Botany,* 96**,** 507-518.

Bailey-Serres, J. & T. D. Colmer (2014) Plant tolerance of flooding stress – recent advances. *Plant, Cell & Environment,* 37**,** 2211-2215.

Bailey-Serres, J., T. Fukao, D. J. Gibbs, M. J. Holdsworth, S. C. Lee, F. Licausi, P. Perata, L. A. C. J. Voesenek & J. T. van Dongen (2012a) Making sense of low oxygen sensing. *Trends in plant science,* 17**,** 129-138.

Bailey-Serres, J., S. C. Lee & E. Brinton (2012b) Waterproofing crops: effective flooding survival strategies. *Plant Physiology,* 160**,** 1698-1709.

Bailey-Serres, J. & L. A. Voesenek (2008) Flooding stress: acclimations and genetic diversity. *Annual Review of Plant Biology,* 59**,** 313-39.

Banti, V., B. Giuntoli, S. Gonzali, E. Loreti, L. Magneschi, G. Novi, E. Paparelli, S. Parlanti, C. Pucciariello & A. Santaniello (2013) Low oxygen response mechanisms in green organisms. *International journal of molecular sciences,* 14**,** 4734-4761.

Banti, V., F. Mafessoni, E. Loreti, A. Alpi & P. Perata (2010) The heat-inducible transcription factor HsfA2 enhances anoxia tolerance in *Arabidopsis*. *Plant Physiology,* 152**,** 1471-1483.

Barrett-Lennard, E. G. (2003) The interaction between waterlogging and salinity in higher plants: causes, consequences and implications. *Plant and Soil,* 253**,** 35-54.

Barrett-Lennard, E. G., P. D. Leighton, F. Buwalda, J. Gibbs, W. Armstrong, C. J. Thomson & H. Greenway (1988) Effects of growing wheat in hypoxic nutrient solutions and of subsequent transfer to aerated solutions. I. growth and carbohydrate status of shoots and roots. *Functional Plant Biology,* 15**,** 585-598.

Barrett-Lennard, E. G. & S. N. Shabala (2013) The waterlogging/salinity interaction in higher plants revisited – focusing on the hypoxia-induced disturbance to K^+ homeostasis. *Functional Plant Biology,* 40**,** 872-882.

Baxter-Burrell, A., Z. Yang, P. S. Springer & J. Bailey-Serres (2002) RopGAP4-Dependent rop GTPase rheostat control of *Arabidopsis* oxygen deprivation tolerance. *Science,* 296**,** 2026-2028.

Baxter, A., R. Mittler & N. Suzuki (2014) ROS as key players in plant stress signalling. *Journal of Experimental Botany,* 65**,** 1229-1240.

Becker, D., S. Hoth, P. Ache, S. Wenkel, M. Roelfsema, O. Meyerhoff, W. Hartung & R. Hedrich (2003) Regulation of the ABA-sensitive *Arabidopsis* potassium channel gene GORK in response to water stress. *FEBS letters,* 554**,** 119-126.

Beckett, P. M., W. Armstrong, S. H. F. W. Justin & J. Armstrong (1988) On the relative importance of convective and diffusive gas flows in plant aeration. *New Phytologist,* 110**,** 463-468.

Begg, C. B. M., G. J. D. Kirk, A. F. Mackenzie & H. U. Neue (1994) Root-induced iron oxidation and pH changes in the lowland rice rhizosphere. *New Phytologist,* 128**,** 469-477.

Benjamin, L. R. & H. Greenway (1979) Effects of a range of O_2 concentrations on porosity of barley roots and on their sugar and protein concentrations. *Annals of Botany,* 43**,** 383-391.

Bertholdsson, N. O., A. Holefors, M. Macaulay & L. A. Crespo-Herrera (2015) QTL for chlorophyll fluorescence of barley plants grown at low oxygen concentration in hydroponics to simulate waterlogging. *Euphytica,* 201**,** 357-365.

Beyer Jr, W. F. & I. Fridovich (1987) Assaying for superoxide dismutase activity: Some large consequences of minor changes in conditions. *Analytical Biochemistry,* 161**,** 559-566.

Beyer, P., S. Al-Babili, X. Ye, P. Lucca, P. Schaub, R. Welsch & I. Potrykus (2002) Golden rice: Introducing the β-carotene biosynthesis pathway into rice endosperm by genetic engineering to defeat vitamin A deficiency. *The Journal of nutrition,* 132**,** 506S-510S.

Blokhina, O., E. Virolainen & K. V. Fagerstedt (2003) Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Annals of Botany,* 91**,** 179-194.

Blum, A. (2005) Drought resistance, water-use efficiency, and yield potential—are they compatible, dissonant, or mutually exclusive? *Australian Journal of Agricultural Research,* 56**,** 1159-1168.

Booker, F. L., U. Blum & E. L. Fiscus (1992) Short-term effects of ferulic acid on ion uptake and water relations in cucumber seedlings. *Journal of Experimental Botany,* 43**,** 649-655.

Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry,* 72**,** 248-254.

Branco-Price, C., K. A. Kaiser, C. J. H. Jang, C. K. Larive & J. Bailey-Serres (2008) Selective mRNA translation coordinates energetic and metabolic adjustments to cellular oxygen deprivation and reoxygenation in *Arabidopsis thaliana*. *The Plant Journal,* 56**,** 743-755.

Broughton, S., G. Zhou, N. Teakle, R. Matsuda, M. Zhou, R. O'Leary, T. Colmer & C. Li (2015) Waterlogging tolerance is associated with root porosity in barley (*Hordeum vulgare* L.). *Molecular Breeding,* 35**,** 1-15.

Brown, J. C. & R. L. Chaney (1971) Effect of iron on the transport of citrate into the xylem of soybeans and tomatoes. *Plant physiology,* 47**,** 836-840.

Bruening, G. & J. Lyons (2000) The case of the FLAVR SAVR tomato. *California Agriculture,* 54**,** 6-7.

Buchanan-Wollaston, V., T. Page, E. Harrison, E. Breeze, P. O. Lim, H. G. Nam, J.-F. Lin, S.-H. Wu, J. Swidzinski, K. Ishizaki & C. J. Leaver (2005) Comparative transcriptome analysis reveals significant differences in gene expression and signalling pathways between developmental and dark/starvation-induced senescence in *Arabidopsis*. *The Plant Journal,* 42**,** 567-585.

Burgos, S., P. Stamp & J. E. Schmid (2001) Agronomic and physiological study of cold and flooding tolerance of spelt (*Triticum spelta* L.) and wheat (*Triticum aestivum* L.). *Journal of Agronomy and Crop Science,* 187**,** 195-202.

Cailliatte, R., A. Schikora, J.-F. Briat, S. Mari & C. Curie (2010) High-affinity manganese uptake by the metal transporter NRAMP1 is essential for *Arabidopsis* growth in low manganese conditions. *The Plant Cell,* 22**,** 904-917.

Carystinos, G. D., H. R. MacDonald, A. F. Monroy, R. S. Dhindsa & R. J. Poole (1995) Vacuolar H^+-translocating pyrophosphatase is induced by anoxia or chilling in seedlings of rice. *Plant Physiology,* 108**,** 641-649.

Cattivelli, L., F. Rizza, F.-W. Badeck, E. Mazzucotelli, A. M. Mastrangelo, E. Francia, C. Mare, A. Tondelli & A. M. Stanca (2008) Drought tolerance improvement in crop plants: An integrated view from breeding to genomics. *Field Crops Research,* 105**,** 1-14.

Chance, B. & A. C. Maehly (1955) Assay of catalases and peroxidases. *Methods in enzymology,* 2**,** 764-775.

Chang, W. W., L. Huang, M. Shen, C. Webster, A. L. Burlingame & J. K. Roberts (2000) Patterns of protein synthesis and tolerance of anoxia in root tips of maize seedlings

acclimated to a low-oxygen environment, and identification of proteins by mass spectrometry. *Plant Physiology,* 122**,** 295-318.

Chardon, F., S. Jasinski, M. Durandet, A. Lécureuil, F. Soulay, M. Bedu, P. Guerche & C. Masclaux-Daubresse (2014) QTL meta-analysis in *Arabidopsis* reveals an interaction between leaf senescence and resource allocation to seeds. *Journal of Experimental Botany,* 65**,** 3949-3962.

Chardon, F., B. Virlon, L. Moreau, M. Falque, J. Joets, L. Decousset, A. Murigneux & A. Charcosset (2004) Genetic architecture of flowering time in maize as inferred from quantitative trait loci meta-analysis and synteny conservation with the rice genome. *Genetics,* 168**,** 2169-2185.

Chaves, M. M., J. Flexas & C. Pinheiro (2009) Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Annals of Botany,* 103**,** 551-560.

Chen, G., T. Krugman, T. Fahima, K. Chen, Y. Hu, M. Roder, E. Nevo & A. Korol (2010) Chromosomal regions controlling seedling drought resistance in Israeli wild barley, *Hordeum spontaneum* C. Koch. *Genetic Resources and Crop Evolution,* 57**,** 85-99.

Chen, Z., Y. Fujii, N. Yamaji, S. Masuda, Y. Takemoto, T. Kamiya, Y. Yusuyin, K. Iwasaki, S.-i. Kato, M. Maeshima, J. F. Ma & D. Ueno (2013) Mn tolerance in rice is mediated by MTP8.1, a member of the cation diffusion facilitator family. *Journal of Experimental Botany,* 64**,** 4375-4387.

Chen, Z., I. Newman, M. Zhou, N. Mendham, G. Zhang & S. Shabala (2005) Screening plants for salt tolerance by measuring K^+ flux: a case study for barley. *Plant, Cell & Environment,* 28**,** 1230-1246.

Chen, Z., I. I. Pottosin, T. A. Cuin, A. T. Fuglsang, M. Tester, D. Jha, I. Zepeda-Jazo, M. Zhou, M. G. Palmgren, I. A. Newman & S. Shabala (2007) Root plasma membrane transporters controlling K^+/Na^+ homeostasis in salt-stressed barley. *Plant Physiology,* 145**,** 1714-1725.

Cheng, N.-h., J. K. Pittman, T. Shigaki & K. D. Hirschi (2002) Characterization of CAX4, an *Arabidopsis* H^+/cation antiporter. *Plant Physiology,* 128**,** 1245-1254.

Christianson, J. A., D. J. Llewellyn, E. S. Dennis & I. W. Wilson (2010) Global gene expression responses to waterlogging in roots and leaves of cotton (*Gossypium hirsutum* l.). *Plant and Cell Physiology,* 51**,** 21-37.

Christianson, J. A., I. W. Wilson, D. J. Llewellyn & E. S. Dennis (2009) The low-oxygen-induced NAC domain transcription factor ANAC102 affects viability of *Arabidopsis* seeds following low-oxygen treatment. *Plant Physiology,* 149**,** 1724-1738.

Clark, R. B. (1982) Plant response to mineral element toxicity and deficiency [Potentially arable soils in the world]. *Breeding plants for less favorable environments*.

Clarkson, D. T. 1988. The uptake and translocation of manganese by plant roots. In *Manganese in soils and plants,* 101-111. Springer.

Colangelo, E. P. & M. L. Guerinot (2004) The essential basic helix-loop-helix protein FIT1 is required for the iron deficiency response. *The Plant Cell,* 16**,** 3400-3412.

Collaku, A. & S. A. Harrison (2005) Heritability of waterlogging tolerance in wheat. *Crop Science,* 45**,** 722–727.

Collard, B. C. Y., M. Z. Z. Jahufer, J. B. Brouwer & E. C. K. Pang (2005) An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica,* 142**,** 169-196.

Collins, N. C., F. o. Tardieu & R. Tuberosa (2008) Quantitative trait loci and crop performance under abiotic stress: where do we stand? *Plant Physiology,* 147**,** 469-486.

Colmer, T. D. (2003a) Aerenchyma and an inducible barrier to radial oxygen loss facilitate root aeration in upland, paddy and deep-water rice (*Oryza sativa* L.). *Annals of Botany,* 91**,** 301-309.

--- (2003b) Long-distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots. *Plant, Cell & Environment,* 26**,** 17-36.

Colmer, T. D., M. C. H. Cox & L. A. C. J. Voesenek (2006) Root aeration in rice (*Oryza sativa)*: evaluation of oxygen, carbon dioxide, and ethylene as possible regulators of root acclimatizations. *New Phytologist,* 170**,** 767-778.

Colmer, T. D. & T. J. Flowers (2008) Flooding tolerance in halophytes. *New Phytologist,* 179**,** 964-974.

Colmer, T. D. & H. Greenway (2010) Ion transport in seminal and adventitious roots of cereals during O_2 deficiency. *Journal of Experimental Botany,* 62**,** 39-57.

Colmer, T. D., R. Munns & T. J. Flowers (2005) Improving salt tolerance of wheat and barley: future prospects. *Aust J Exp Agr,* 45**,** 1425–1443.

Colmer, T. D. & L. A. C. J. Voesenek (2009) Flooding tolerance: suites of plant traits in variable environments. *Functional Plant Biology,* 36**,** 665-681.

Curie, C., J. M. Alonso, L. Marie, J. R. Ecker & J.-F. Briat (2000) Involvement of NRAMP1 from *Arabidopsis thaliana* in iron transport. *Biochemical Journal,* 347**,** 749-755.

Curie, C., Z. Panaviene, C. Loulergue, S. L. Dellaporta, J.-F. Briat & E. L. Walker (2001) Maize *yellow stripe1* encodes a membrane protein directly involved in Fe(III) uptake. *Nature,* 409**,** 346-349.

Damanik, R., M. Maziah, M. Ismail, S. Ahmad & A. Zain (2010) Responses of the antioxidative enzymes in Malaysian rice (*Oryza sativa* L.) cultivars under submergence condition. *Acta Physiologiae Plantarum,* 32**,** 739-747.

Darvasi, A. & M. Soller (1997) A simple method to calculate resolving power and confidence interval of QTL map location. *Behavior Genetics,* 27**,** 125-132.

de Dorlodot, S., B. Forster, L. Pagès, A. Price, R. Tuberosa & X. Draye (2007) Root system architecture: opportunities and constraints for genetic improvement of crops. *Trends in Plant Science,* 12**,** 474-481.

Delhaize, E., B. D. Gruber, J. K. Pittman, R. G. White, H. Leung, Y. Miao, L. Jiang, P. R. Ryan & A. E. Richardson (2007) A role for the AtMTP11 gene of *Arabidopsis* in manganese transport and tolerance. *The Plant Journal,* 51**,** 198-210.

Delhaize, E., T. Kataoka, D. M. Hebb, R. G. White & P. R. Ryan (2003) Genes encoding proteins of the cation diffusion facilitator family that confer manganese tolerance. *The Plant Cell,* 15**,** 1131-1142.

Demidchik, V. & F. Maathuis. 2010. *Ion channels and plant stress responses*. Springer.

Demidchik, V., S. N. Shabala & J. M. Davies (2007) Spatial variation in H_2O_2 response of *Arabidopsis thaliana* root epidermal Ca^{2+} flux and plasma membrane Ca^{2+} channels. *The Plant Journal,* 49**,** 377-386.

Demidchik, V., D. Straltsova, S. S. Medvedev, G. A. Pozhvanov, A. Sokolik & V. Yurin (2014) Stress-induced electrolyte leakage: the role of K^+-permeable channels and involvement in programmed cell death and metabolic adjustment. *Journal of Experimental Botany,* 65**,** 1259-1270.

Diab, A., B. Teulat-Merah, D. This, N. Ozturk, D. Benscher & M. Sorrells (2004) Identification of drought-inducible genes and differentially expressed sequence tags in barley. *Theoretical and Applied Genetics,* 109**,** 1417-1425.

Dietz, K. J., N. Tavakoli, C. Kluge, T. Mimura, S. S. Sharma, G. C. Harris, A. N. Chardonnens & D. Golldack (2001) Significance of the V-type ATPase for the adaptation to stressful growth conditions and its regulation on the molecular and biochemical level. *Journal of Experimental Botany,* 52**,** 1969-1980.

Drew, M. & J. M. Lynch (1980) Soil anaerobiosis, microorganisms, and root function. *Annual Review of Phytopathology,* 18**,** 37-66.
Drew, M. C. (1997) Oxygen deficiency and root metabolism: injury and acclimation under hypoxia and anoxia. *Annual review of plant biology,* 48**,** 223-250.
Drew, M. C., M. B. Jackson, S. C. Giffard & R. Campbell (1981) Inhibition by silver ions of gas space (aerenchyma) formation in adventitious roots of *Zea mays* L. subjected to exogenous ethylene or to oxygen deficiency. *Planta,* 153**,** 217-224.
Drew, M. C., P. H. Saglio & A. Pradet (1985) Larger adenylate energy charge and ATP/ADP ratios in aerenchymatous roots of *Zea mays* in anaerobic media as a consequence of improved internal oxygen transport. *Planta,* 165**,** 51-58.
Drew, M. C. & E. J. Sisworo (1977) Early effects of flooding on nitrogen deficiency and leaf chlorosis in barley. *New Phytologist,* 79**,** 567-571.
Dreyer, I. & N. Uozumi (2011) Potassium channels in plant cells. *FEBS Journal,* 278**,** 4293-4303.
Durrett, T. P., W. Gassmann & E. E. Rogers (2007) The FRD3-mediated efflux of citrate into the root vasculature is necessary for efficient iron translocation. *Plant physiology,* 144**,** 197-205.
Eagles, H. A., H. S. Bariana, F. C. Ogbonnaya, G. J. Rebetzke, G. J. Hollamby, R. J. Henry, P. H. Henschke & M. Carter (2001) Implementation of markers in Australian wheat breeding. *Australian Journal of Agricultural Research,* 52**,** 1349–1356.
El-Jaoual, T. & D. A. Cox (1998) Manganese toxicity in plants. *Journal of Plant Nutrition,* 21**,** 353-386.
Evans, D. E. (2004) Aerenchyma formation. *New Phytologist,* 161**,** 35-49.
Evans, H. J. & G. J. Sorger (1966) Role of mineral elements with emphasis on the univalent cations. *Annual review of plant physiology,* 17**,** 47-76.
Fageria, N., A. Santos, M. Barbosa Filho & C. Guimarães (2008) Iron toxicity in lowland rice. *Journal of plant nutrition,* 31**,** 1676-1697.
Fagerstedt, K. V. 2010. Programmed Cell Death and Aerenchyma Formation Under Hypoxia. In *Waterlogging signalling and tolerance in plants,* eds. S. Mancuso & S. Shabala, 99-118. Heidelberg, Germany: Springer-Verlag.
Fan, Y., S. Shabala, Y. Ma, R. Xu & M. Zhou (2015) Using QTL mapping to investigate the relationships between abiotic stress tolerance (drought and salinity) and agronomic and physiological traits. *BMC genomics,* 16**,** 43.
Fan, Y., M. Zhu, S. Shabala, C. D. Li, P. Johnson & M. X. Zhou (2014) Antioxidant activity in salt-stressed barley leaves: evaluating time- and age-dependence and suitability for the use as a biochemical marker in breeding programs. *Journal of Agronomy and Crop Science,* 200**,** 261-272.
Fang, W.-C. & C. H. Kao (2000) Enhanced peroxidase activity in rice leaves in response to excess iron, copper and zinc. *Plant Science,* 158**,** 71-76.
Fecht-Christoffers, M. M., H.-P. Braun, C. Lemaitre-Guillier, A. VanDorsselaer & W. J. Horst (2003) Effect of manganese toxicity on the proteome of the leaf apoplast in cowpea. *Plant physiology,* 133**,** 1935-1946.
Felle, H. H. (2005) pH regulation in anoxic plants. *Annals of Botany,* 96**,** 519-532.
Fiedler, S., M. J. Vepraskas, J. L. Richardson & L. S. Donald. 2007. Soil redox potential: Importance, field measurements, and observations. In *Advances in Agronomy,* 1-54. Academic Press.
Fleck, A. T., T. Nye, C. Repenning, F. Stahl, M. Zahn & M. K. Schenk (2011) Silicon enhances suberization and lignification in roots of rice (*Oryza sativa*). *Journal of Experimental Botany,* 62**,** 2001-2011.

Flowers, T. J. & T. D. Colmer (2008) Salinity tolerance in halophytes. *New Phytologist,* 179**,** 945-963.

Flowers, T. J. & S. A. Flowers (2005) Why does salinity pose such a difficult problem for plant breeders? *Agricultural Water Management,* 78**,** 15-24.

Foy, C., R. t. Chaney & M. White (1978) The physiology of metal toxicity in plants. *Annual Review of Plant Physiology,* 29**,** 511-566.

Gambrell, R. P., R. D. DeLaune & W. H. Patrick Jr (1991) Redox processes in soils following oxygen depletion. *Plant Life Under Oxygen Deprivation. SPB Academic Publishing BV, The Hague, The Netherlands***,** 101-117.

Garthwaite, A. J., W. Armstrong & T. D. Colmer (2008) Assessment of O_2 diffusivity across the barrier to radial O_2 loss in adventitious roots of *Hordeum marinum*. *New Phytologist,* 179**,** 405-416.

Garthwaite, A. J., E. Steudle & T. D. Colmer (2006) Water uptake by roots of *Hordeum marinum*: formation of a barrier to radial O_2 loss does not affect root hydraulic conductivity. *Journal of Experimental Botany,* 57**,** 655-664.

Garthwaite, A. J., R. von Bothmer & T. D. Colmer (2003) Diversity in root aeration traits associated with waterlogging tolerance in the genus *Hordeum*. *Functional Plant Biology,* 30**,** 875 - 889.

Gechev, T. S., F. Van Breusegem, J. M. Stone, I. Denev & C. Laloi (2006) Reactive oxygen species as signals that modulate plant stress responses and programmed cell death. *BioEssays,* 28**,** 1091-1101.

Geisler-Lee, J., C. Caldwell & D. R. Gallie (2010) Expression of the ethylene biosynthetic machinery in maize roots is regulated in response to hypoxia. *Journal of Experimental Botany,* 61**,** 857-871.

Gibberd, M. R., T. D. Colmer & P. S. Cocks (1999) Root porosity and oxygen movement in waterlogging-tolerant *Trifolium tomentosum* and -intolerant *Trifolium glomeratum*. *Plant, Cell and Environment* 22**,** 1161-1168.

Gibberd, M. R., J. D. Gray, P. S. Cocks & T. D. Colmer (2001) Waterlogging tolerance among a diverse range of *Trifolium* accessions is related to root porosity, lateral root formation and `aerotropic rooting'. *Annals of Botany,* 88**,** 579-589.

Gibbs, D. J., S. C. Lee, N. Md Isa, S. Gramuglia, T. Fukao, G. W. Bassel, C. S. Correia, F. Corbineau, F. L. Theodoulou, J. Bailey-Serres & M. J. Holdsworth (2011) Homeostatic response to hypoxia is regulated by the N-end rule pathway in plants. *Nature,* 479**,** 415-418.

Gibbs, J. & H. Greenway (2003) Review: Mechanisms of anoxia tolerance in plants. I. Growth, survival and anaerobic catabolism. *Functional Plant Biology,* 30**,** 353-353.

Gill, S. S. & N. Tuteja (2010) Polyamines and abiotic stress tolerance in plants. *Plant Signaling & Behavior,* 5**,** 26-33.

Glass, A. D. (1973) Influence of phenolic acids on ion uptake I. Inhibition of phosphate uptake. *Plant physiology,* 51**,** 1037-1041.

Glass, A. D. M. (1974) Influence of Phenolic Acids upon Ion Uptake: III. INHIBITION OF POTASSIUM ABSOBPTION. *Journal of Experimental Botany,* 25**,** 1104-1113.

Goffinet, B. & S. Gerber (2000) Quantitative trait loci: A Meta-analysis. *Genetics,* 155**,** 463-473.

Green, L. S. & E. E. Rogers (2004) FRD3 controls iron localization in *Arabidopsis*. *Plant Physiology,* 136**,** 2523-2531.

Greenway, H., W. Armstrong & T. D. Colmer (2006) Conditions Leading to High CO_2 (>5 kPa) in Waterlogged–Flooded Soils and Possible Effects on Root Growth and Metabolism. *Annals of Botany,* 98**,** 9-32.

Greenway, H. & J. Gibbs (2003) Review: Mechanisms of anoxia tolerance in plants. II. Energy requirements for maintenance and energy distribution to essential processes. *Functional Plant Biology,* 30**,** 999-1036.

Guerinot, M. L. & Y. Yi (1994) Iron: nutritious, noxious, and not readily available. *Plant Physiology,* 104**,** 815-820.

Guo, L., H. Yang, X. Zhang & S. Yang (2013) *Lipid transfer protein 3* as a target of *MYB96* mediates freezing and drought stress in *Arabidopsis*. *Journal of Experimental Botany,* 64**,** 1755-1767.

Guo, P., M. Baum, R. Varshney, A. Graner, S. Grando & S. Ceccarelli (2008) QTLs for chlorophyll and chlorophyll fluorescence parameters in barley under post-flowering drought. *Euphytica,* 163**,** 203-214.

Hall, J. L. (2002) Cellular mechanisms for heavy metal detoxification and tolerance. *Journal of Experimental Botany,* 53**,** 1-11.

Haque, E., A. Oyanagi & K. Kawaguchi (2012) Aerenchyma formation in the seminal roots of Japanese wheat cultivars in relation to growth under waterlogged conditions. *Plant Production Science,* 15**,** 164-173.

Haroldsen, V., G. Paulino, C. Chi-ham & A. Bennett (2012) Research and adoption of biotechnology strategies could improve California fruit and nut crops. *California Agriculture,* 66**,** 62-69.

Hattori, Y., K. Nagai & M. Ashikari (2011) Rice growth adapting to deepwater. *Current Opinion in Plant Biology,* 14**,** 100-105.

He, C.-j., S. A. Finlayson, M. C. Drew, W. R. Jordan & P. W. Morgan (1996a) Ethylene biosynthesis during aerenchyma formation in roots of maize subjected to mechanical impedance and hypoxia. *Plant Physiology,* 112**,** 1679-1685.

He, C.-J., P. W. Morgan & M. C. Drew (1996b) Transduction of an ethylene signal is required for cell death and lysis in the root cortex of maize during aerenchyma formation induced by hypoxia. *Plant Physiology,* 112**,** 463-472.

He, Y., J. Zhang, H. Ma, Y. Tu, J. Chen, F. Chen, H. Xu, Z. Song, D. Cai & X. Xue (2012) Aerenchyma formation and increased accumulation of free proline in roots of xerophytic *Aloe vera* L. cultured in nutrient solutions. *Journal of Medicinal Plants Research,* 6**,** 243-252.

Hell, R. & U. W. Stephan (2003) Iron uptake, trafficking and homeostasis in plants. *Planta,* 216**,** 541-551.

Henriques, R., J. Jásik, M. Klein, E. Martinoia, U. Feller, J. Schell, M. S. Pais & C. Koncz (2002) Knock-out of *Arabidopsis* metal transporter gene *IRT1* results in iron deficiency accompanied by cell differentiation defects. *Plant Molecular Biology,* 50**,** 587-597.

Horiguchi, T. (1988) Mechanism of manganese toxicity and tolerance of plants: IV. Effects of silicon on alleviation of manganese toxicity of rice plants. *Soil Science and Plant Nutrition,* 34**,** 65-73.

Horst, W. 1988. The physiology of manganese toxicity. In *Manganese in soils and plants,* 175-188. Springer.

Hossain, M. A., P. Piyatida, J. A. T. da Silva & M. Fujita (2012) Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. *Journal of Botany,* 2012.

Hossain, Z., M. F. Lopez-Climent, V. Arbona, R. M. Perez-Clemente & A. Gomez-Cadenas (2009) Modulation of the antioxidant system in citrus under waterlogging and subsequent drainage. *Journal of Plant Physiology,* 166**,** 1391-1404.

Hu, H. & L. Xiong (2014) Genetic engineering and breeding of drought-resistant crops. *Annual Review of Plant Biology,* 65**,** 715-741.

Huang, X., S. Shabala, L. Shabala, Z. Rengel, X. Wu, G. Zhang & M. Zhou (2014) Linking waterlogging tolerance with Mn^{2+} toxicity: a case study for barley. *Plant Biology,* 17**,** 26-33.

Inoue, H., D. Mizuno, M. Takahashi, H. Nakanishi, S. Mori & N. K. Nishizawa (2004) A rice FRD3-like (*OsFRDL1*) gene is expressed in the cells involved in long-distance transport. *Soil Science and Plant Nutrition,* 50**,** 1133-1140.

Ishimaru, Y., H. Masuda, K. Bashir, H. Inoue, T. Tsukamoto, M. Takahashi, H. Nakanishi, N. Aoki, T. Hirose, R. Ohsugi & N. K. Nishizawa (2010) Rice metal-nicotianamine transporter, OsYSL2, is required for the long-distance transport of iron and manganese. *The Plant Journal,* 62**,** 379-390.

Ishimaru, Y., M. Suzuki, T. Tsukamoto, K. Suzuki, M. Nakazono, T. Kobayashi, Y. Wada, S. Watanabe, S. Matsuhashi, M. Takahashi, H. Nakanishi, S. Mori & N. K. Nishizawa (2006) Rice plants take up iron as an Fe^{3+}-phytosiderophore and as Fe^{2+}. *The Plant Journal,* 45**,** 335-346.

Ishimaru, Y., R. Takahashi, K. Bashir, H. Shimo, T. Senoura, K. Sugimoto, K. Ono, M. Yano, S. Ishikawa & T. Arao (2012) Characterizing the role of rice *NRAMP5* in manganese, iron and cadmium transport. *Scientific reports,* 2**,** 286.

Izaguirre-Mayoral, M. L. & T. R. Sinclair (2005) Variation in manganese and iron accumulation among soybean genotypes growing on hydroponic solutions of differing manganese and nitrate concentrations. *Journal of plant nutrition,* 28**,** 521-535.

Jackson, M., C. Dobson, B. Herman & A. Merryweather (1984) Modification of 3,5-diiodo-4-hydroxybenzoic acid (DIHB) activity and stimulation of ethylene production by small concentrations of oxygen in the root environment. *Plant Growth Regulation,* 2**,** 251-262.

Jackson, M. B. (2002) Long-distance signalling from roots to shoots assessed: the flooding story. *Journal of Experimental Botany,* 53**,** 175-181.

Jackson, M. B. & W. Armstrong (1999) Formation of aerenchyma and the processes of plant ventilation in relation to soil flooding and submergence. *Plant Biology,* 1**,** 274-287.

Jackson, M. B., T. M. Fenning, M. C. Drew & L. R. Saker (1985) Stimulation of ethylene production and gas-space (aerenchyma) formation in adventitious roots of *Zea mays* L. by small partial pressures of oxygen. *Planta,* 165**,** 486-492.

Jackson, M. B. & K. C. Hall (1987) Early stomatal closure in waterlogged pea plants is mediated by abscisic acid in the absence of foliar water deficits. *Plant, Cell and Environment,* 10**,** 121-130.

Jaeger, C., A. Gessler, S. Biller, H. Rennenberg & J. Kreuzwieser (2009) Differences in C metabolism of ash species and provenances as a consequence of root oxygen deprivation by waterlogging. *Journal of Experimental Botany.*

Jan, A., K. Maruyama, D. Todaka, S. Kidokoro, M. Abo, E. Yoshimura, K. Shinozaki, K. Nakashima & K. Yamaguchi-Shinozaki (2013) OsTZF1, a CCCH-tandem zinc finger protein, confers delayed senescence and stress tolerance in rice by regulating stress-related genes. *Plant physiology,* 161**,** 1202-1216.

Jiang, Z., X.-F. Song, Z.-Q. Zhou, L.-K. Wang, J.-W. Li, X.-Y. Deng & H.-Y. Fan (2010) Aerenchyma formation: programmed cell death in adventitious roots of winter wheat (*Triticum aestivum*) under waterlogging. *Functional Plant Biology,* 37**,** 748–755.

John, C. D. (1977) The structure of rice roots grown in aerobic and anaerobic environments. *Plant and Soil,* 47**,** 269-274.

Jones, H. G. (2007) Monitoring plant and soil water status: established and novel methods revisited and their relevance to studies of drought tolerance. *Journal of Experimental Botany,* 58**,** 119-130.

Joshi, R., A. Shukla, S. C. Mani & P. Kumar (2010) Hypoxia induced non-apoptotic cellular changes during aerenchyma formation in rice (*Oryza sativa* L.) roots. *Physiology and Molecular Biology of Plants,* 16**,** 99-106.

Jung, J., S. Lee & H.-K. Choi (2008) Anatomical patterns of aerenchyma in aquatic and wetland plants. *Journal of Plant Biology,* 51**,** 428-439.

Justin, S. H. F. W. & W. Armstrong (1987) The Anatomical Characteristics of Roots and Plant Response to Soil Flooding. *New Phytologist,* 106**,** 465-495.

Karuppanapandian, T., J.-C. Moon, C. Kim, K. Manoharan & W. Kim (2011) Reactive oxygen species in plants: their generation, signal transduction, and scavenging mechanisms. *Australian Journal of Crop Science,* 5**,** 709-725.

Kawase, M. & R. E. Whitmoyer (1980) Aerenchyma development in waterlogged plants. *American Journal of Botany,* 67**,** 18-22.

Kearsey, M. J. (1998) The principles of QTL analysis (a minimal mathematics approach). *Journal of Experimental Botany,* 49**,** 1619-1623.

Kemp, P. J. & C. Peers (2007) Oxygen sensing by ion channels. *Essays in biochemistry,* 43**,** 77-90.

Kenward, K. D., J. Brandle, J. McPherson & P. L. Davies (1999) Type II fish antifreeze protein accumulation in transgenic tobacco does not confer frost resistance. *Transgenic research,* 8**,** 105-117.

Khabaz-Saberi, H., S. Barker & Z. Rengel (2012) Tolerance to ion toxicities enhances wheat (*Triticum aestivum* L.) grain yield in waterlogged acidic soils. *Plant and soil,* 354**,** 371-381.

Khabaz-Saberi, H. & Z. Rengel (2010) Aluminum, manganese, and iron tolerance improves performance of wheat genotypes in waterlogged acidic soils. *Journal of Plant Nutrition and Soil Science,* 173**,** 461-468.

Khabaz-Saberi, H., T. L. Setter & I. Waters (2005) Waterlogging induces high to toxic concentrations of iron, aluminum, and manganese in wheat varieties on acidic soil. *Journal of Plant Nutrition,* 29**,** 899-911.

Khowaja, F., G. Norton, B. Courtois & A. Price (2009) Improved resolution in the position of drought-related QTLs in a single mapping population of rice by meta-analysis. *BMC Genomics,* 10**,** 276.

Khush, G. S. (2001) Green revolution: the way forward. *Nature Reviews Genetics,* 2**,** 815-822.

Kirk, G. J. D. & J. B. Bajita (1995) Root-induced iron oxidation, pH changes and zinc solubilization in the rhizosphere of lowland rice. *New Phytologist,* 131**,** 129-137.

Kirk, G. J. D., J. L. Solivas & M. C. Alberto (2003) Effects of flooding and redox conditions on solute diffusion in soil. *European Journal of Soil Science,* 54**,** 617-624.

Koike, S., H. Inoue, D. Mizuno, M. Takahashi, H. Nakanishi, S. Mori & N. K. Nishizawa (2004) OsYSL2 is a rice metal-nicotianamine transporter that is regulated by iron and expressed in the phloem. *The Plant Journal,* 39**,** 415-424.

Koizumi, Y., Y. Hara, Y. Yazaki, K. Sakano & K. Ishizawa (2011) Involvement of plasma membrane H^+-ATPase in anoxic elongation of stems in pondweed (*Potamogeton distinctus*) turions. *New Phytologist,* 190**,** 421-430.

Korff, M., S. Grando, A. Del Greco, D. This, M. Baum & S. Ceccarelli (2008) Quantitative trait loci associated with adaptation to Mediterranean dryland conditions in barley. *Theoretical and Applied Genetics,* 117**,** 653-669.

Kotula, L., T. D. Colmer & M. Nakazono (2014) Effects of organic acids on the formation of the barrier to radial oxygen loss in roots of *Hordeum marinum*. *Functional Plant Biology,* 41**,** 187-202.

Kotula, L., K. Ranathunge, L. Schreiber & E. Steudle (2009a) Functional and chemical comparison of apoplastic barriers to radial oxygen loss in roots of rice (*Oryza sativa* L.) grown in aerated or deoxygenated solution. *Journal of Experimental Botany,* 60**,** 2155-2167.

Kotula, L., K. Ranathunge & E. Steudle (2009b) Apoplastic barriers effectively block oxygen permeability across outer cell layers of rice roots under deoxygenated conditions: roles of apoplastic pores and of respiration. *New Phytologist,* 184**,** 909-917.

Kotula, L. & E. Steudle (2009) Measurements of oxygen permeability coefficients of rice (*Oryza sativa* L.) roots using a new perfusion technique. *Journal of Experimental Botany,* 60**,** 567-580.

Kreuzwieser, J., S. Fürniss & H. Rennenberg (2002) Impact of waterlogging on the N-metabolism of flood tolerant and non-tolerant tree species. *Plant, Cell & Environment,* 25**,** 1039-1049.

Kreuzwieser, J., J. Hauberg, K. A. Howell, A. Carroll, H. Rennenberg, A. H. Millar & J. Whelan (2009) Differential response of gray poplar leaves and roots underpins stress adaptation during hypoxia. *Plant Physiology,* 149**,** 461-473.

Kumutha, D., K. Ezhilmathi, R. K. Sairam, G. C. Srivastava, P. S. Deshmukh & R. C. Meena (2009) Waterlogging induced oxidative stress and antioxidant activity in pigeonpea genotypes. *Biologia Plantarum,* 53**,** 75-84.

Laohavisit, A. & J. M. Davies (2007) The gas that opens gates: calcium channel activation by ethylene. *New Phytologist,* 174**,** 470-473.

Laurie, S. H., N. P. Tancock, S. P. McGrath & J. R. Sanders (1995) Influence of EDTA complexation on plant uptake of manganese (II). *Plant Science,* 109**,** 231-235.

Lee, T. G., C. S. Jang, J. Y. Kim, D. S. Kim, J. H. Park, D. Y. Kim & Y. W. Seo (2007) A Myb transcription factor (*TaMyb1*) from wheat roots is expressed during hypoxia: roles in response to the oxygen concentration in root environment and abiotic stresses. *Physiologia Plantarum,* 129**,** 375-385.

Lee, Y.-H., K.-S. Kim, Y.-S. Jang, J.-H. Hwang, D.-H. Lee & I.-H. Choi (2014) Global gene expression responses to waterlogging in leaves of rape seedlings. *Plant Cell Reports,* 33**,** 289-299.

Li, H., R. Vaillancourt, N. Mendham & M. Zhou (2008) Comparative mapping of quantitative trait loci associated with waterlogging tolerance in barley (*Hordeum vulgare* L.). *BMC Genomics,* 9**,** 401.

Li, W.-T., C. Liu, Y.-X. Liu, Z.-E. Pu, S.-F. Dai, J.-R. Wang, X.-J. Lan, Y.-L. Zheng & Y.-M. Wei (2013) Meta-analysis of QTL associated with tolerance to abiotic stresses in barley. *Euphytica,* 189**,** 31-49.

Licausi, F. (2011) Regulation of the molecular response to oxygen limitations in plants. *New Phytologist,* 190**,** 550-555.

Licausi, F., M. Kosmacz, D. A. Weits, B. Giuntoli, F. M. Giorgi, L. A. C. J. Voesenek, P. Perata & J. T. van Dongen (2011) Oxygen sensing in plants is mediated by an N-end rule pathway for protein destabilization. *Nature,* 479**,** 419-422.

Licausi, F., C. Pucciariello & P. Perata (2013) New Role for an Old Rule: N-end Rule-Mediated Degradation of Ethylene Responsive Factor Proteins Governs Low Oxygen Response in PlantsF. *Journal of Integrative Plant Biology,* 55**,** 31-39.

Luo, X., X. Bai, D. Zhu, Y. Li, W. Ji, H. Cai, J. Wu, B. Liu & Y. Zhu (2012) GsZFP1, a new Cys2/His2-type zinc-finger protein, is a positive regulator of plant tolerance to cold and drought stress. *Planta,* 235**,** 1141-1155.

Lynch, J. M. (1977) Phytotoxicity of acetic acid produced in the anaerobic decomposition of wheat straw. *Journal of Applied Bacteriology,* 42**,** 81-87.
Ma, Y., S. Shabala, C. Li, C. Liu, W. Zhang & M. Zhou (2015) Quantitative trait loci for salinity tolerance identified under drained and waterlogged conditions and their association with flowering time in barley (*Hordeum vulgare*. L). *PLoS ONE,* 10**,** e0134822.
Macnair, M. R., G. H. Tilstone & S. E. Smith (1999) 13 The Genetics of Metal Tolerance and Accumulation in Higher Plants. *Phytoremediation of contaminated soil and water***,** 235.
Malik, A. I., T. D. Colmer, H. Lambers & M. Schortemeyer (2001) Changes in physiological and morphological traits of roots and shoots of wheat in response to different depths of waterlogging. *Australian Journal of Plant Physiology,* 28**,** 1121-1131.
Malik, A. I., T. D. Colmer, H. Lambers & M. Schortemeyer (2003) Aerenchyma formation and radial O_2 loss along adventitious roots of wheat with only the apical root portion exposed to O_2 deficiency. *Plant, Cell and Environment,* 26**,** 1713-1722.
Malik, A. I., T. D. Colmer, H. Lambers, T. L. Setter & M. Schortemeyer (2002) Short-term waterlogging has long-term effects on the growth and physiology of wheat. *New Phytologist,* 153**,** 225-236.
Malik, A. I., J. P. English & T. D. Colmer (2009) Tolerance of *Hordeum marinum* accessions to O_2 deficiency, salinity and these stresses combined. *Annals of Botany,* 103**,** 237-48.
Malik, A. I., A. K. M. R. Islam & T. D. Colmer (2011) Transfer of the barrier to radial oxygen loss in roots of *Hordeum marinum* to wheat (*Triticum aestivum*): evaluation of four *H. marinum*–wheat amphiploids. *New Phytologist,* 190**,** 499-508.
Mano, Y. & F. Omori (2008) Verification of QTL controlling root aerenchyma formation in a maize × teosinte "*Zea nicaraguensis*" advanced backcross population. *Breeding Science,* 58**,** 217-223.
--- (2009) High-density linkage map around the root aerenchyma locus Qaer1.06 in the backcross populations of maize Mi29 × teosinte "*Zea nicaraguensis*". *Breeding Science,* 59**,** 427-433.
Mano, Y. & F. Omori (2013) Flooding tolerance in interspecific introgression lines containing chromosome segments from teosinte (*Zea nicaraguensis*) in maize (*Zea mays* subsp. *mays*). *Annals of Botany,* 112**,** 1125-1139.
Mano, Y. & F. Omori (2015) Flooding tolerance in maize (*Zea mays* subsp. *mays*) F1 hybrids containing a QTL introgressed from teosinte (*Zea nicaraguensis*). *Euphytica,* 205**,** 255-267.
Mano, Y., F. Omori, B. Kindiger & H. Takahashi (2008) A linkage map of maize maize × teosinte *Zea luxurians* and identification of QTLs controlling root aerenchyma formation. *Molecular Breeding,* 21**,** 327-337.
Mano, Y., F. Omori, M. Muraki & T. Takamizo (2005) QTL Mapping of Adventitious Root Formation under Flooding Conditions in Tropical Maize (*Zea mays* L.) Seedlings. *Breeding Science,* 55**,** 343-347
Mano, Y., F. Omori, T. Takamizo, B. Kindiger, R. M. Bird & C. H. Loaisiga (2006) Variation for root aerenchyma formation in flooded and non-flooded maize and teosinte seedlings. *Plant and Soil,* 281**,** 269-279.
Mano, Y., F. Omori, T. Takamizo, B. Kindiger, R. M. Bird, C. H. Loaisiga & H. Takahashi (2007) QTL mapping of root aerenchyma formation in seedlings of a maize × rare teosinte "*Zea nicaraguensis*" cross. *Plant and Soil,* 295**,** 103-113.
Mano, Y. & K. Takeda (1997) Mapping quantitative trait loci for salt tolerance at germination and the seedling stage in barley (*Hordeum vulgare* L.). *Euphytica,* 94**,** 263-272.

Mano, Y. & K. Takeda (2012) Accurate evaluation and verification of varietal ranking for flooding tolerance at the seedling stage in barley (*Hordeum vulgare* L.). *Breeding Science,* 62**,** 3-10.

Marschner, H. (1995) Mineral nutrition of higher plants. 2nd. *Edn. Academic Pres*.

Mcdonald, M. P., N. W. Galwey & T. D. Colmer (2001) Waterlogging tolerance in the tribe Triticeae: the adventitious roots of *Critesion marinum* have a relatively high porosity and a barrier to radial oxygen loss. *Plant, Cell and Environment,* 24**,** 585-596.

Menegus, F., L. Cattaruzza, M. Mattana, N. Beffagna & E. Ragg (1991) Response to anoxia in rice and wheat seedlings changes in the pH of intracellular compartments, glucose-6-phosphate level, and metabolic rate. *Plant Physiology,* 95**,** 760-767.

Mino, Y., T. Ishida, N. Ota, M. Inoue, K. Nomoto, T. Takemoto, H. Tanaka & Y. Sugiura (1983) Mugineic acid-iron(III) complex and its structurally analogous cobalt(III) complex: characterization and implication for absorption and transport of iron in gramineous plants. *Journal of the American Chemical Society,* 105**,** 4671-4676.

Mittler, R. (2006) Abiotic stress, the field environment and stress combination. *Trends in Plant Science,* 11**,** 15-19.

Moellenbeck, D. J., M. L. Peters, J. W. Bing, J. R. Rouse, L. S. Higgins, L. Sims, T. Nevshemal, L. Marshall, R. T. Ellis & P. G. Bystrak (2001) Insecticidal proteins from *Bacillus thuringiensis* protect corn from corn rootworms. *Nature biotechnology,* 19**,** 668-672.

Møller, I. M., P. E. Jensen & A. Hansson (2007) Oxidative modifications to cellular components in plants. *Annu. Rev. Plant Biol.,* 58**,** 459-481.

Mugnai, S., A. M. Marras & S. Mancuso (2011) Effect of Hypoxic Acclimation on Anoxia Tolerance in *Vitis* Roots: Response of Metabolic Activity and K^+ Fluxes. *Plant and Cell Physiology,* 52**,** 1107-1116.

Muhlenbock, P., M. Plaszczyca, M. Plaszczyca, E. Mellerowicz & S. Karpinski (2007) Lysigenous aerenchyma formation in *Arabidopsis* is controlled by *LESION SIMULATING DISEASE1*. *The Plant Cell,* 19**,** 3819–3830.

Munns, R. (2005) Genes and salt tolerance: bringing them together. *New Phytologist,* 167**,** 645-663.

Munns, R., R. A. James, B. Xu, A. Athman, S. J. Conn, C. Jordans, C. S. Byrt, R. A. Hare, S. D. Tyerman, M. Tester, D. Plett & M. Gilliham (2012) Wheat grain yield on saline soils is improved by an ancestral Na^+ transporter gene. *Nat Biotech,* 30**,** 360-364.

Munns, R. & M. Tester (2008) Mechanisms of salinity tolerance. *Annual Review of Plant Biology,* 59**,** 651-681.

Murata, Y., J. F. Ma, N. Yamaji, D. Ueno, K. Nomoto & T. Iwashita (2006) A specific transporter for iron(III)–phytosiderophore in barley roots. *The Plant Journal,* 46**,** 563-572.

Nakano, Y. & K. Asada (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and Cell Physiology,* 22**,** 867-880.

Nandi, S., P. K. Subudhi, D. Senadhira, N. L. Manigbas, S. Sen-Mandi & N. Huang (1997) Mapping QTLs for submergence tolerance in rice by AFLP analysis and selective genotyping. *Molecular and General Genetics MGG,* 255**,** 1-8.

Nevo, Y. & N. Nelson (2006) The NRAMP family of metal-ion transporters. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research,* 1763**,** 609-620.

Nguyen, V., S. Ribot, O. Dolstra, R. Niks, R. F. Visser & C. G. van der Linden (2013) Identification of quantitative trait loci for ion homeostasis and salt tolerance in barley (*Hordeum vulgare* L.). *Molecular Breeding,* 31**,** 137-152.

Nishiuchi, S., T. Yamauchi, H. Takahashi, L. Kotula & M. Nakazono (2012) Mechanisms for coping with submergence and waterlogging in rice. *Rice,* 5**,** 2.

Nozoye, T., S. Nagasaka, T. Kobayashi, M. Takahashi, Y. Sato, Y. Sato, N. Uozumi, H. Nakanishi & N. K. Nishizawa (2011) Phytosiderophore efflux transporters are crucial for iron acquisition in graminaceous plants. *Journal of Biological Chemistry,* 286**,** 5446-5454.

Ohkawa, H., H. Tsujii & Y. Ohkawa (1999) The use of cytochrome P450 genes to introduce herbicide tolerance in crops: a review. *Pesticide Science,* 55**,** 867-874.

Overmyer, K., M. Brosche & J. Kangasjarvi (2003) Reactive oxygen species and hormonal control of cell death. *Trends in plant science,* 8**,** 335-342.

P de San Celedonio, R., L. G. Abeledo & D. Miralles (2014) Identifying the critical period for waterlogging on yield and its components in wheat and barley. *Plant and Soil,* 378**,** 265-277.

Pang, J., T. Cuin, L. Shabala, M. Zhou, N. Mendham & S. Shabala (2007a) Effect of secondary metabolites associated with anaerobic soil conditions on ion fluxes and electrophysiology in barley roots. *Plant Physiology,* 145**,** 266-76.

Pang, J., J. Ross, M. Zhou, N. Mendham & S. Shabala (2007b) Amelioration of detrimental effects of waterlogging by foliar nutrient sprays in barley. *Functional Plant Biology,* 34**,** 221-227.

Pang, J. & S. Shabala. 2010. Membrane Transporters and Waterlogging Tolerance. In *Waterlogging signalling and tolerance in plants,* eds. S. Mancuso & S. Shabala, 197-219. Heidelberg, Germany: Springer-Verlag.

Pang, J., M. Zhou, N. Mendham & S. Shabala (2004) Growth and physiological responses of six barley genotypes to waterlogging and subsequent recovery. *Australian Journal of Agricultural Research,* 55**,** 895-906.

Pang, J. Y., I. A. N. Newman, N. Mendham, M. Zhou & S. Shabala (2006) Microelectrode ion and O_2 fluxes measurements reveal differential sensitivity of barley root tissues to hypoxia. *Plant, Cell and Environment,* 29**,** 1107-1121.

Paterson, A. H., J. W. DeVerna, B. Lanini & S. D. Tanksley (1990) Fine mapping of quantitative trait loci using selected overlapping recombinant chromosomes, in an interspecies cross of tomato. *Genetics,* 124**,** 735-742.

Pedas, P., C. K. Ytting, A. T. Fuglsang, T. P. Jahn, J. K. Schjoerring & S. Husted (2008) Manganese efficiency in barley: identification and characterization of the metal ion transporter HvIRT1. *Plant physiology,* 148**,** 455-466.

Pedersen, O., S. M. Rich & T. D. Colmer (2009) Surviving floods: leaf gas films improve O_2 and CO_2 exchange, root aeration, and growth of completely submerged rice. *The Plant Journal,* 58**,** 147-156.

Perata, P., W. Armstrong & L. A. C. J. Voesenek (2011) Plants and flooding stress. *New Phytologist,* 190**,** 269-273.

Peschke, V. M. & M. M. Sachs (1994) Characterization and expression of transcripts induced by oxygen deprivation in maize (*Zea mays* L.). *Plant Physiology,* 104**,** 387-394.

Pezeshki, S. R. (2001) Wetland plant responses to soil flooding. *Environmental and Experimental Botany,* 46**,** 299-312.

Pezeshki, S. R. & R. D. DeLaune (1998) Responses of seedlings of selected woody species to soil oxidation-reduction conditions. *Environmental and Experimental Botany,* 40**,** 123-133.

Pittman, J. K. (2005) Managing the manganese: molecular mechanisms of manganese transport and homeostasis. *New Phytologist,* 167**,** 733-742.

Qi, X.-H., X.-W. Xu, X.-J. Lin, W.-J. Zhang & X.-H. Chen (2012) Identification of differentially expressed genes in cucumber (*Cucumis sativus* L.) root under waterlogging stress by digital gene expression profile. *Genomics,* 99**,** 160-168.

Qin, F., K. Shinozaki & K. Yamaguchi-Shinozaki (2011) Achievements and challenges in understanding plant abiotic stress responses and tolerance. *Plant & Cell Physiology,* 52**,** 1569-1582.

Qiu, F., Y. Zheng, Z. Zhang & S. Xu (2007) Mapping of QTL associated with waterlogging tolerance during the seedling stage in maize. *Annals of Botany,* 99**,** 1067-81.

Rabello, A. R., C. M. Guimarães, P. H. Rangel, F. R. da Silva, D. Seixas, E. de Souza, A. C. Brasileiro, C. R. Spehar, M. E. Ferreira & Â. Mehta (2008) Identification of drought-responsive genes in roots of upland rice (*Oryza sativa* L). *BMC Genomics,* 9**,** 1-13.

Rajhi, I., T. Yamauchi, H. Takahashi, S. Nishiuchi, K. Shiono, R. Watanabe, A. Mliki, Y. Nagamura, N. Tsutsumi, N. K. Nishizawa & M. Nakazono (2011) Identification of genes expressed in maize root cortical cells during lysigenous aerenchyma formation using laser microdissection and microarray analyses. *New Phytologist,* 190**,** 351-68.

Ranathunge, K., L. Kotula, E. Steudle & R. Lafitte (2004) Water permeability and reflection coefficient of the outer part of young rice roots are differently affected by closure of water channels (aquaporins) or blockage of apoplastic pores. *Journal of Experimental Botany,* 55**,** 433-447.

Ranathunge, K., J. Lin, E. Steudle & L. Schreiber (2011) Stagnant deoxygenated growth enhances root suberization and lignifications, but differentially affects water and NaCl permeabilities in rice (*Oryza sativa* L.) roots. *Plant, Cell and Environment,* 34**,** 1223-1240.

Raskin, I. (1983) A method for measuring leaf volume, density, thickness, and internal gas volume. *Hortscience,* 18**,** 698-699.

Ratcliffe, R. G. (1997) In *vivo* NMR studies of the metabolic response of plant tissues to anoxia. *Annals of Botany,* 79**,** 39-48.

Raven, J. A. (2008) Not drowning but photosynthesizing: probing plant plastrons. *New Phytologist,* 177**,** 841-845.

Reddy, K. R. & W. H. Patrick jr (1976) Effect of frequent changes in aerobic and anaerobic conditions on redox potential and nitrogen loss in a flooded soil. *Soil Biology and Biochemistry,* 8**,** 491-495.

Reggiani, R., S. Zaina & A. Bertani (1992) Plasmalemma ATPase in rice coleoptiles; Stimulation by putrescine and polyamines. *Phytochemistry,* 31**,** 417-419.

Rellán-Álvarez, R., J. Giner-Martínez-Sierra, J. Orduna, I. Orera, J. Á. Rodríguez-Castrillón, J. I. García-Alonso, J. Abadía & A. Álvarez-Fernández (2010) Identification of a Tri-Iron(III), Tri-Citrate Complex in the Xylem Sap of Iron-Deficient Tomato Resupplied with Iron: New Insights into Plant Iron Long-Distance Transport. *Plant and Cell Physiology,* 51**,** 91-102.

Rengel, Z. (1999) Manganese uptake and transport in plants. *Metal ions in biological systems,* 37**,** 57-87.

Rivandi, J., J. Miyazaki, M. Hrmova, M. Pallotta, M. Tester & N. C. Collins (2011) A *SOS3* homologue maps to *HvNax4*, a barley locus controlling an environmentally sensitive Na+ exclusion trait. *Journal of Experimental Botany,* 62**,** 1201-1216.

Rivoal, J. & A. D. Hanson (1993) Evidence for a large and sustained glycolytic flux to lactate in anoxic roots of some members of the halophytic genus *Limonium*. *Plant Physiology,* 101**,** 553-560.

Robinson, N. J., C. M. Procter, E. L. Connolly & M. L. Guerinot (1999) A ferric-chelate reductase for iron uptake from soils. *Nature,* 397**,** 694-697.

Rocha, M., F. Licausi, W. L. Araújo, A. Nunes-Nesi, L. Sodek, A. R. Fernie & J. T. van Dongen (2010) Glycolysis and the tricarboxylic acid cycle are linked by alanine aminotransferase during hypoxia induced by waterlogging of *Lotus japonicus*. *Plant Physiology,* 152**,** 1501-1513.

Rogers, E. E. & M. L. Guerinot (2002) FRD3, a member of the multidrug and toxin efflux family, controls iron deficiency responses in *Arabidopsis*. *The Plant Cell,* 14**,** 1787-1799.

Roy, S. J., E. J. Tucker & M. Tester (2011) Genetic analysis of abiotic stress tolerance in crops. *Current Opinion in Plant Biology,* 14**,** 232-239.

Ryan, P. R., S. D. Tyerman, T. Sasaki, T. Furuichi, Y. Yamamoto, W. H. Zhang & E. Delhaize (2011) The identification of aluminium-resistance genes provides opportunities for enhancing crop production on acid soils. *Journal of Experimental Botany,* 62**,** 9-20.

Saab, I. N. & M. M. Sachs (1996) A flooding-induced xyloglucan *Endo*-Transglycosylase homolog in maize is responsive to ethylene and associated with aerenchyma. *Plant Physiology,* 112**,** 385-391.

Said, J., M. Song, H. Wang, Z. Lin, X. Zhang, D. Fang & J. Zhang (2015) A comparative meta-analysis of QTL between intraspecific *Gossypium hirsutum* and interspecific *G. hirsutum* × *G. barbadense* populations. *Molecular Genetics and Genomics,* 290**,** 1003-1025.

Sairam, R. K., D. Kumutha, K. Ezhilmathi, V. Chinnusamy & R. C. Meena (2009) Waterlogging induced oxidative stress and antioxidant enzyme activities in pigeon pea. *Biologia Plantarum,* 53**,** 493-504.

Sairam, R. K., D. Kumutha, K. Ezhilmathi, P. S. Deshmukh & G. C. Srivastava (2008) Physiology and biochemistry of waterlogging tolerance in plants. *Biologia Plantarum* 52**,** 401-412.

Sairam, R. K. & G. C. Srivastava (2001) Water stress tolerance of wheat (*Triticum aestivum* L.): variations in hydrogen peroxide accumulation and antioxidant activity in tolerant and susceptible genotypes. *Journal of Agronomy and Crop Science,* 186**,** 63-70.

Salvi, S. & R. Tuberosa (2005) To clone or not to clone plant QTLs: present and future challenges. *Trends in Plant Science,* 10**,** 297-304.

Sasaki, A., N. Yamaji, J. Xia & J. F. Ma (2011) OsYSL6 is involved in the detoxification of excess manganese in rice. *Plant Physiology,* 157**,** 1832-1840.

Sasaki, A., N. Yamaji, K. Yokosho & J. F. Ma (2012) *Nramp5* is a major transporter responsible for manganese and cadmium uptake in rice. *The Plant Cell,* 24**,** 2155-2167.

Sayed, M., H. Schumann, K. Pillen, A. Naz & J. Leon (2012) AB-QTL analysis reveals new alleles associated to proline accumulation and leaf wilting under drought stress conditions in barley (*Hordeum vulgare* L.). *BMC Genetics,* 13**,** 61.

Schaaf, G., E. Catoni, M. Fitz, R. Schwacke, A. Schneider, N. Von Wiren & W. Frommer (2002) A putative role for the vacuolar calcium/manganese proton antiporter AtCAX2 in heavy metal detoxification. *Plant Biology,* 4**,** 612-618.

Schaaf, G., B. E. Erenoglu & N. von Wirén (2004) Physiological and biochemical characterization of metal-phytosiderophore transport in graminaceous species. *Soil Science and Plant Nutrition,* 50**,** 989-995.

Seago, J., L. Marsh, K. Stevens, A. Soukup, O. Votrubova & D. E. Enstone (2005) A re-examination of the root cortex in wetland flowering plants with respect to aerenchyma. *Annals of Botany,* 96**,** 565-579.

Semagn, K., Y. Beyene, M. Warburton, A. Tarekegne, S. Mugo, B. Meisel, P. Sehabiague & B. Prasanna (2013) Meta-analyses of QTL for grain yield and anthesis silking interval in 18 maize populations evaluated under water-stressed and well-watered environments. *BMC Genomics,* 14**,** 313.

Septiningsih, E. M., A. M. Pamplona, D. L. Sanchez, C. N. Neeraja, G. V. Vergara, S. Heuer, A. M. Ismail & D. J. Mackill (2009) Development of submergence-tolerant rice cultivars: the *Sub1* locus and beyond. *Annals of Botany,* 103**,** 151–160.

Setter, T. L., P. Burgess, I. Waters & J. Kuo. 1999. Genetic diversity of barley and wheat for waterlogging tolerance in Western Australia. In *Australian Barley Technical Symposium*.

Setter, T. L. & I. Waters (2003) Review of prospects for germplasm improvement for waterlogging tolerance in wheat, barley and oats. *Plant and Soil,* 253**,** 1-34.

Setter, T. L., I. Waters, S. K. Sharma, K. N. Singh, N. Kulshreshtha, N. P. Yaduvanshi, P. C. Ram, B. N. Singh, J. Rane, G. McDonald, H. Khabaz-Saberi, T. B. Biddulph, R. Wilson, I. Barclay, R. McLean & M. Cakir (2009) Review of wheat improvement for waterlogging tolerance in Australia and India: the importance of anaerobiosis and element toxicities associated with different soils. *Annals of Botany,* 103**,** 221-35.

Shabala, L., T. Ross, T. McMeekin & S. Shabala (2006) Non-invasive microelectrode ion flux measurements to study adaptive responses of microorganisms to the environment. *FEMS Microbiology Reviews,* 30**,** 472-486.

Shabala, S. (2011) Physiological and cellular aspects of phytotoxicity tolerance in plants: the role of membrane transporters and implications for crop breeding for waterlogging tolerance. *New Phytologist,* 190**,** 289-298.

Shabala, S. & J. Bose. 2012. Application of Non-invasive Microelectrode Flux Measurements in Plant Stress Physiology. In *Plant Electrophysiology: Methods and Cell Electrophysiology,* ed. A. G. Volkov, 91-126. Berlin, Heidelberg: Springer Berlin Heidelberg.

Shabala, S., T. A. Cuin, L. Prismall & L. G. Nemchinov (2007) Expression of animal CED-9 anti-apoptotic gene in tobacco modifies plasma membrane ion fluxes in response to salinity and oxidative stress. *Planta,* 227**,** 189-197.

Shabala, S. & I. Pottosin (2014) Regulation of potassium transport in plants under hostile conditions: implications for abiotic and biotic stress tolerance. *Physiologia Plantarum,* 151**,** 257-279.

Shabala, S., L. Shabala, J. Barcelo & C. Poschenrieder (2014) Membrane transporters mediating root signalling and adaptive responses to oxygen deprivation and soil flooding. *Plant, Cell & Environment,* 37**,** 2216-2233.

Shabala, S., L. Shabala & I. Newman. 2012. Studying Membrane Transport Processes by Non-invasive Microelectrodes: Basic Principles and Methods. In *Plant Electrophysiology: Methods and Cell Electrophysiology,* ed. A. G. Volkov, 167-186. Berlin, Heidelberg: Springer Berlin Heidelberg.

Shabala, S., S. Shabala, T. A. Cuin, J. Pang, W. Percey, Z. Chen, S. Conn, C. Eing & L. H. Wegner (2010) Xylem ionic relations and salinity tolerance in barley. *The Plant Journal,* 61**,** 839-853.

Sharma, S. S. & K.-J. Dietz (2009) The relationship between metal toxicity and cellular redox imbalance. *Trends in Plant Science,* 14**,** 43-50.

Shavrukov, Y., N. Gupta, J. Miyazaki, M. Baho, K. Chalmers, M. Tester, P. Langridge & N. Collins (2010) *HvNax3*—a locus controlling shoot sodium exclusion derived from wild barley (*Hordeum vulgare* ssp. *spontaneum*). *Functional & Integrative Genomics,* 10**,** 277-291.

Shaw, R. E., W. S. Meyer, A. McNeill & S. D. Tyerman (2013) Waterlogging in Australian agricultural landscapes: a review of plant responses and crop models. *Crop and Pasture Science,* 64**,** 549-562.

Shigaki, T., J. K. Pittman & K. D. Hirschi (2003) Manganese Specificity Determinants in the *Arabidopsis*Metal/H^+ Antiporter CAX2. *Journal of Biological Chemistry,* 278**,** 6610-6617.

Shimamura, S., R. Yamamoto, T. Nakamura, S. Shimada & S. Komatsu (2010) Stem hypertrophic lenticels and secondary aerenchyma enable oxygen transport to roots of soybean in flooded soil. *Annals of Botany,* 106**,** 277-284.

Shimamura, S., S. Yoshida & T. Mochizuki (2007) Cortical aerenchyma formation in hypocotyl and adventitious roots of *Luffa cylindrica* subjected to soil flooding. *Annals of Botany,* 100**,** 1431-1439.

Shiono, K., S. Ogawa, S. Yamazaki, H. Isoda, T. Fujimura, M. Nakazono & T. D. Colmer (2011) Contrasting dynamics of radial O_2-loss barrier induction and aerenchyma formation in rice roots of two lengths. *Annals of Botany,* 107**,** 89-99.

Shiono, K., H. Takahashi, T. D. Colmer & M. Nakazono (2008) Role of ethylene in acclimations to promote oxygen transport in roots of plants in waterlogged soils. *Plant Science,* 175**,** 52-58.

Siangliw, M., T. Toojinda, S. Tragoonrung & A. Vanavichit (2003) Thai Jasmine Rice Carrying QTLch9 (SubQTL) is Submergence Tolerant. *Annals of Botany,* 91**,** 255-261.

Singh, S., D. J. Mackill & A. M. Ismail (2009) Responses of *SUB1* rice introgression lines to submergence in the field: Yield and grain quality. *Field Crops Research,* 113**,** 12-23.

Sosnowski, O., A. Charcosset & J. Joets (2012) BioMercator V3: an upgrade of genetic map compilation and quantitative trait loci meta-analysis algorithms. *Bioinformatics,* 28**,** 2082-2083.

Starkey, R. L. (1966) Oxidation and reduction of sulfur compounds in soils. *Soil Science,* 101**,** 297-306.

Steffens, B., T. Geske & M. Sauter (2010) Aerenchyma formation in the rice stem and its promotion by H_2O_2. *New Phytologist,* 190**,** 369-378.

Striker, G. G., P. Insausti, A. A. Grimoldi & A. S. Vega (2007) Trade-off between root porosity and mechanical strength in species with different types of aerenchyma. *Plant Cell and Environment,* 30**,** 580-589.

Suralta, R. R. & A. Yamauchi (2008) Root growth, aerenchyma development, and oxygen transport in rice genotypes subjected to drought and waterlogging. *Environmental and Experimental Botany,* 64**,** 75-82.

Swamy, B. P. M., P. Vikram, S. Dixit, H. U. Ahmed & A. Kumar (2011) Meta-analysis of grain yield QTL identified during agricultural drought in grasses showed consensus. *BMC Genomics,* 12**,** 319.

Tan, W., J. Liu, T. Dai, Q. Jing, W. Cao & D. Jiang (2008) Alterations in photosynthesis and antioxidant enzyme activity in winter wheat subjected to post-anthesis water-logging. *Photosynthetica,* 46**,** 21-27.

Tang, B., S.-z. Xu, X.-l. Zou, Y.-l. Zheng & F.-z. Qiu (2010) Changes of antioxidative enzymes and lipid peroxidation in leaves and roots of waterlogging-tolerant and waterlogging-sensitive maize genotypes at seedling stage. *Agricultural Sciences in China,* 9**,** 651-661.

Teakle, N. L., J. Armstrong, E. G. Barrett-Lennard & T. D. Colmer (2011) Aerenchymatous phellem in hypocotyl and roots enables O_2 transport in *Melilotus siculus*. *New Phytologist,* 190**,** 340-350.

Teakle, N. L., N. Bazihizina, S. Shabala, T. D. Colmer, E. G. Barrett-Lennard, A. Rodrigo-Moreno & A. E. Läuchli (2013) Differential tolerance to combined salinity and O_2 deficiency in the halophytic grasses *Puccinellia* ciliata and *Thinopyrum ponticum*:

The importance of K^+ retention in roots. *Environmental and Experimental Botany,* 87**,** 69-78.
Tester, M. & P. Langridge (2010) Breeding technologies to increase crop production in a changing world. *Science,* 327**,** 818-822.
Teulat, B., C. Borries & D. This (2001) New QTLs identified for plant water status, water-soluble carbohydrate and osmotic adjustment in a barley population grown in a growth-chamber under two water regimes. *Theoretical and Applied Genetics,* 103**,** 161-170.
Teulat, B., D. This, M. Khairallah, C. Borries, C. Ragot, P. Sourdille, P. Leroy, P. Monneveux & A. Charrier (1998) Several QTLs involved in osmotic-adjustment trait variation in barley (*Hordeum vulgare* L.). *Theoretical and Applied Genetics,* 96**,** 688-698.
Teulat, B., N. Zoumarou-Wallis, B. Rotter, M. Ben Salem, H. Bahri & D. This (2003) QTL for relative water content in field-grown barley and their stability across Mediterranean environments. *Theoretical and Applied Genetics,* 108**,** 181-188.
This, D., C. Borries, I. Souyris & B. Teulat (2000) QTL study of chlorophyll content as a genetic parameter of drought tolerance in barley. *Barley genetics newsletter,* 30**,** 20-23.
Thomas, A. L., S. M. C. Guerreiro & L. Sodek (2005) Aerenchyma formation and recovery from hypoxia of the flooded root system of nodulated soybean. *Annals of Botany,* 96**,** 1191-1198.
Thomson, C. J., W. Armstrong, I. Waters & H. Greenway (1990) Aerenchyma formation and associated oxygen movement in seminal and nodal roots of wheat. *Plant, Cell & Environment,* 13**,** 395-403.
Tiffin, L. O. (1966) Iron Translocation I. Plant Culture, Exudate Sampling, Iron-Citrate Analysis. *Plant Physiology,* 41**,** 510-514.
Torres, M. A. & J. L. Dangl (2005) Functions of the respiratory burst oxidase in biotic interactions, abiotic stress and development. *Current Opinion in Plant Biology,* 8**,** 397-403.
Trought, M. C. T. & M. C. Drew (1980) The development of waterlogging damage in wheat seedlings (*Triticum aestivum* L.). *Plant and Soil,* 54**,** 77-94.
Tsukamoto, T., H. Nakanishi, S. Kiyomiya, S. Watanabe, S. Matsuhashi, N. K. Nishizawa & S. Mori (2006) 52Mn translocation in barley monitored using a positron-emitting tracer imaging system. *Soil science and plant nutrition,* 52**,** 717-725.
Tsukamoto, T., H. Nakanishi, H. Uchida, S. Watanabe, S. Matsuhashi, S. Mori & N. K. Nishizawa (2009) 52Fe Translocation in Barley as Monitored by a Positron-Emitting Tracer Imaging System (PETIS): Evidence for the Direct Translocation of Fe from Roots to Young Leaves via Phloem. *Plant and Cell Physiology,* 50**,** 48-57.
Tuberosa, R. (2012) Phenotyping for drought tolerance of crops in the genomics era. *Frontiers in Physiology,* 3**,** 347.
Tuberosa, R. & S. Salvi (2006) Genomics-based approaches to improve drought tolerance of crops. *Trends in Plant Science,* 11**,** 405-412.
Unger, I. M., P. P. Motavalli & R.-M. Muzika (2009) Changes in soil chemical properties with flooding: A field laboratory approach. *Agriculture, Ecosystems & Environment,* 131**,** 105-110.
Ushimaru, T., S. Kanematsu, M. Katayama & H. Tsuji (2001) Antioxidative enzymes in seedlings of *Nelumbo nucifera* germinated under water. *Physiologia Plantarum,* 112**,** 39-46.

Ushimaru, T., S. Kanematsu, M. Shibasaka & H. Tsuji (1999) Effect of hypoxia on the antioxidative enzymes in aerobically grown rice (*Oryza sativa*) seedlings. *Physiologia Plantarum,* 107**,** 181-187.

Van Ooijen, J. W. & B. V. Kyazma (2009) MapQTL 6. *Software for the mapping of quantitative trait loci in experimental populations of diploid species. Kyazma BV: Wageningen, Netherlands*.

Varotto, C., D. Maiwald, P. Pesaresi, P. Jahns, F. Salamini & D. Leister (2002) The metal ion transporter IRT1 is necessary for iron homeostasis and efficient photosynthesis in *Arabidopsis thaliana*. *The Plant Journal,* 31**,** 589-599.

Vert, G., M. Barberon, E. Zelazny, M. Séguéla, J.-F. Briat & C. Curie (2009) *Arabidopsis* IRT2 cooperates with the high-affinity iron uptake system to maintain iron homeostasis in root epidermal cells. *Planta,* 229**,** 1171-1179.

Vert, G., J.-F. Briat & C. Curie (2001) *Arabidopsis IRT2* gene encodes a root-periphery iron transporter. *The Plant Journal,* 26**,** 181-189.

Vert, G., N. Grotz, F. Dédaldéchamp, F. Gaymard, M. L. Guerinot, J.-F. Briat & C. Curie (2002) IRT1, an *Arabidopsis* transporter essential for iron uptake from the soil and for plant growth. *The Plant Cell,* 14**,** 1223-1233.

Veyrieras, J.-B., B. Goffinet & A. Charcosset (2007) MetaQTL: a package of new computational methods for the meta-analysis of QTL mapping experiments. *BMC Bioinformatics,* 8**,** 49.

Visser, E. J. W. & G. M. Bögemann (2003) Measurement of porosity in very small samples of plant tissue. *Plant and Soil,* 253**,** 81-90.

Visser, E. J. W. & G. M. Bögemann (2006) Aerenchyma formation in the wetland plant *Juncus effusus* is independent of ethylene. *New Phytologist,* 171**,** 305-314.

Visser, E. J. W., T. D. Colmer, C. W. P. M. Blom & L. A. C. J. Voesenek (2000) Changes in growth, porosity, and radial oxygen loss from adventitious roots of selected mono- and dicotyledonous wetland species with contrasting types of aerenchyma. *Plant, Cell and Environment,* 23**,** 1237-1245.

Voesenek, L. A. C. J., W. Armstrong, ouml, G. M. gemann, T. D. Colmer & M. P. McDonald (1999) A lack of aerenchyma and high rates of radial oxygen loss from the root base contribute to the waterlogging intolerance of *Brassica napus*. *Functional Plant Biology,* 26**,** 87-93.

Voesenek, L. A. C. J., T. D. Colmer, R. Pierik, F. F. Millenaar & A. J. M. Peeters (2006) How plants cope with complete submergence. *New Phytologist,* 170**,** 213-226.

Voesenek, L. A. C. J. & R. Sasidharan (2013) Ethylene – and oxygen signalling – drive plant survival during flooding. *Plant Biology,* 15**,** 426-435.

Voesenek, L. A. C. J., R. Sasidharan, E. J. W. Visser & J. Bailey-Serres (2016) Flooding stress signaling through perturbations in oxygen, ethylene, nitric oxide and light. *New Phytologist,* 209**,** 39-43.

Voesenek, L. A. C. J., H. van Veen & R. Sasidharan (2014) Learning from nature: the use of non-model species to identify novel acclimations to flooding stress. *AoB Plants,* 6.

Voorrips, R. E. (2002) MapChart: Software for the Graphical Presentation of Linkage Maps and QTLs. *Journal of Heredity,* 93**,** 77-78.

Wang, F., W. Tong, H. Zhu, W. Kong, R. Peng, Q. Liu & Q. Yao (2016a) A novel Cys2/His2 zinc finger protein gene from sweetpotato, IbZFP1, is involved in salt and drought tolerance in transgenic Arabidopsis. *Planta,* 243**,** 783-797.

Wang, K. & Y. Jiang (2007) Antioxidant responses of creeping bentgrass roots to waterlogging. *Crop Science,* 47**,** 232-238.

Wang, T., T. Tohge, A. A. Ivakov, B. Mueller-Roeber, A. R. Fernie, M. Mutwil, J. H. Schippers & S. Persson (2015) Salt-Related MYB1 (SRM1) coordinates abscisic acid

biosynthesis and signaling during salt stress in *Arabidopsis*. *Plant Physiology,* 169**,** 1027–1041.

Wang, Y., J. Xu, D. Deng, H. Ding, Y. Bian, Z. Yin, Y. Wu, B. Zhou & Y. Zhao (2016b) A comprehensive meta-analysis of plant morphology, yield, stay-green, and virus disease resistance QTL in maize (*Zea mays* L.). *Planta,* 243**,** 459-471.

Wang, Y. X., P. Wu, Y. R. Wu & X. L. Yan (2002) Molecular marker analysis of manganese toxicity tolerance in rice under greenhouse conditions. *Plant and Soil,* 238**,** 227-233.

Ward, J. M. (2001) Identification of novel families of membrane proteins from the model plant *Arabidopsis thaliana*. *Bioinformatics,* 17**,** 560-563.

Watson, E. R., P. Lapins & R. J. W. Barron (1976) Effect of waterlogging on the growth, grain and straw yield of wheat, barley and oats. *Australian Journal of Experimental Agriculture and Animal Husbandry 16(78) 114 - 122,* 16**,** 114-122.

Webb, J. & M. B. Jackson (1986) AtTransmission and cryo-scanning electron microscopy study of the formation of aerenchyma (cortical gas-filled space) in adventitious roots of rice (*Oryza sativa*). *Journal of Experimental Botany,* 37**,** 832-841.

Wegner, L. H. 2010. Oxygen Transport in Waterlogged Plants. In *Waterlogging signalling and tolerance in plants,* eds. S. Mancuso & S. Shabala, 3-22. Heidelberg, Germany: Springer-Verlag.

William, H. M., R. Trethowan & E. M. Crosby-Galvan (2007) Wheat breeding assisted by markers: CIMMYT's experience. *Euphytica,* 157**,** 307-319.

Wong, H. L., T. Sakamoto, T. Kawasaki, K. Umemura & K. Shimamoto (2004) Down-regulation of Metallothionein, a reactive oxygen scavenger, by the small GTPase OsRac1 in rice. *Plant Physiology,* 135**,** 1447–1456.

Xia, J.-H. & P. H. Saglio (1992) Lactic acid efflux as a mechanism of hypoxic acclimation of maize root tips to anoxia. *Plant Physiology,* 100**,** 40-46.

Xu, G.-Y., P. C. F. Rocha, M.-L. Wang, M.-L. Xu, Y.-C. Cui, L.-Y. Li, Y.-X. Zhu & X. Xia (2011) A novel rice calmodulin-like gene, *OsMSR2,* enhances drought and salt tolerance and increases ABA sensitivity in *Arabidopsis*. *Planta,* 234**,** 47-59.

Xu, K. & D. Mackill (1996) A major locus for submergence tolerance mapped on rice chromosome 9. *Molecular Breeding,* 2**,** 219-224.

Xu, K., X. Xu, T. Fukao, P. Canlas, R. Maghirang-Rodriguez, S. Heuer, A. M. Ismail, J. Bailey-Serres, P. C. Ronald & D. J. Mackill (2006) *Sub1A* is an ethylene-response-factor-like gene that confers submergence tolerance to rice. *Nature,* 442**,** 705-708.

Xu, K., X. Xu, P. C. Ronald & D. J. Mackill (2000) A high-resolution linkage map of the vicinity of the rice submergence tolerance locus *Sub1*. *Molecular and General Genetics MGG,* 263**,** 681-689.

Xu, R., J. Wang, C. Li, P. Johnson, C. Lu & M. Zhou (2012) A single locus is responsible for salinity tolerance in a Chinese landrace barley (*Hordeum vulgare* L.). *PLoS One,* 7**,** e43079.

Xu, Y. & J. H. Crouch (2008) Marker-Assisted selection in plant breeding: from publications to practice. *Crop Science,* 48**,** 391-407.

Xue, D. W., M. X. Zhou, X. Q. Zhang, S. Chen, K. Wei, F. R. Zeng, Y. Mao, F. B. Wu & G. P. Zhang (2010) Identification of QTLs for yield and yield components of barley under different growth conditions. *Journal of Zhejiang University SCIENCE B,* 11**,** 169-76.

Yamauchi, T., F. Abe, K. Kawaguchi, A. Oyanagi & M. Nakazono (2014a) Adventitious roots of wheat seedlings that emerge in oxygen-deficient conditions have increased root diameters with highly developed lysigenous aerenchyma. *Plant Signaling & Behavior,* 9**,** e28506.

Yamauchi, T., S. Shimamura, M. Nakazono & T. Mochizuki (2013) Aerenchyma formation in crop species: A review. *Field Crops Research,* 152**,** 8-16.

Yamauchi, T., A. Tanaka, H. Mori, I. Takamure, K. Kato & M. Nakazono (2016) Ethylene-dependent aerenchyma formation in adventitious roots is regulated differently in rice and maize. *Plant, Cell & Environment,* 39**,** 2145-2157.

Yamauchi, T., K. Watanabe, A. Fukazawa, H. Mori, F. Abe, K. Kawaguchi, A. Oyanagi & M. Nakazono (2014b) Ethylene and reactive oxygen species are involved in root aerenchyma formation and adaptation of wheat seedlings to oxygen-deficient conditions. *Journal of Experimental Botany,* 65**,** 261-273.

Yan, B., Q. Dai, X. Liu, S. Huang & Z. Wang (1996) Flooding-induced membrane damage, lipid oxidation and activated oxygen generation in corn leaves. *Plant and Soil,* 179**,** 261-268.

Yin, D., S. Chen, F. Chen & J. Jiang (2013) Ethylene promotes induction of aerenchyma formation and ethanolic fermentation in waterlogged roots of *Dendranthema* spp. *Molecular Biology Reports,* 40**,** 4581-4590.

Yokosho, K., N. Yamaji, D. Ueno, N. Mitani & J. F. Ma (2009) OsFRDL1 is a citrate transporter required for efficient translocation of iron in rice. *Plant physiology,* 149**,** 297-305.

Yokotani, N., T. Ichikawa, Y. Kondou, M. Iwabuchi, M. Matsui, H. Hirochika & K. Oda (2013) Role of the rice transcription factor JAmyb in abiotic stress response. *Journal of Plant Research,* 126**,** 131-139.

Yordanova, R. Y., K. N. Christov & L. P. Popova (2004) Antioxidative enzymes in barley plants subjected to soil flooding. *Environmental and Experimental Botany,* 51**,** 93-101.

Yordanova, R. Y. & L. P. Popova (2001) Photosynthetic response of barley plants to soil flooding. *Photosynthetica,* 39**,** 515-520.

Yordanova, R. Y., A. N. Uzunova & L. P. Popova (2005) Effects of short-term soil flooding on stomata behaviour and leaf gas exchange in barley plants. *Biologia Plantarum,* 49**,** 317-319.

Young, N. D., D. Zamir, M. W. Ganal & S. D. Tanksley (1988) Use of isogenic lines and simultaneous probing to identify DNA markers tightly linked to the tm-2a gene in tomato. *Genetics,* 120**,** 579-585.

Yu, F., X. Han, C. Geng, Y. Zhao, Z. Zhang & F. Qiu (2015) Comparative proteomic analysis revealing the complex network associated with waterlogging stress in maize (*Zea mays* L.) seedling root cells. *PROTEOMICS,* 15**,** 135-147.

Yu, Q. & Z. Rengel (1999) Waterlogging influences plant growth and activities of superoxide dismutases in narrow-leafed lupin and transgenic tobacco plants. *Journal of Plant Physiology,* 155**,** 431-438.

Zaharieva, T. & V. Römheld (2000) Specific Fe^{2+} uptake system in strategy I plants inducible under Fe deficiency. *Journal of Plant Nutrition,* 23**,** 1733-1744.

Zeng, F., L. Shabala, M. Zhou, G. Zhang & S. Shabala (2013) Barley responses to combined waterlogging and salinity stress: separating effects of oxygen deprivation and elemental toxicity. *Frontiers in plant science,* 4**,** 1-13.

Zhang, D., J. Tong, Z. Xu, P. Wei, L. Xu, Q. Wan, Y. Huang, X. He, J. Yang, H. Shao & H. Ma (2016a) Soybean C2H2-Type zinc finger protein *GmZFP3* with conserved QALGGH motif negatively regulates drought responses in transgenic *Arabidopsis*. *Frontiers in Plant Science,* 7**,** 325.

Zhang, F., G. Chen, Q. Huang, O. Orion, T. Krugman, T. Fahima, A. B. Korol, E. Nevo & Y. Gutterman (2005) Genetic basis of barley caryopsis dormancy and seedling

desiccation tolerance at the germination stage. *Theoretical and Applied Genetics,* 110**,** 445-453.
Zhang, G., K. Tanakamaru, J. Abe & S. Morita (2007) Influence of waterlogging on some anti-oxidative enzymatic activities of two barley genotypes differing in anoxia tolerance. *Acta Physiologiae Plantarum,* 29**,** 171-176.
Zhang, H.-X. & E. Blumwald (2001) Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit. *Nat Biotech,* 19**,** 765-768.
Zhang, J., T. Van Toai, L. Huynh & J. Preiszner (2000) Development of flooding-tolerant *Arabidopsis thaliana* by autoregulated cytokinin production. *Molecular Breeding,* 6**,** 135-144.
Zhang, J. & X. Zhang (1994) Can early wilting of old leaves account for much of the ABA accumulation in flooded pea plants? *Journal of Experimental Botany,* 45**,** 1335-1342.
Zhang, L.-Y., D.-C. Liu, X.-L. Guo, W.-L. Yang, J.-Z. Sun, D.-W. Wang & A. Zhang (2010) Genomic distribution of quantitative trait loci for yield and yield-related traits in common wheat. *Journal of Integrative Plant Biology,* 52**,** 996-1007.
Zhang, X., X. Huang, M. Zhou, L. Shabala, A. Koutoulis & S. Shabala. 2015a. Plant Breeding for Flood Tolerance: Advances and Limitations. In *Genetic Manipulation in Plants for Mitigation of Climate Change*, 43-72. Springer.
Zhang, X., S. Shabala, A. Koutoulis, L. Shabala, P. Johnson, D. Hayes, D. Nichols & M. Zhou (2015b) Waterlogging tolerance in barley is associated with faster aerenchyma formation in adventitious roots. *Plant and Soil,* 394**,** 355-372.
Zhang, X., S. Shabala, A. Koutoulis, L. Shabala & M. Zhou (2016b) Meta-analysis of major QTL for abiotic stress tolerance in barley and implications for barley breeding. *Planta***,** 1-13.
Zhang, X., G. Zhou, S. Shabala, A. Koutoulis, L. Shabala, P. Johnson, C. Li & M. Zhou (2016c) Identification of aerenchyma formation-related QTL in barley that can be effective in breeding for waterlogging tolerance. *Theoretical and Applied Genetics,* 129**,** 1167-1177.
--- (2016d) Identification of aerenchyma formation-related QTL in barley that can be effective in breeding for waterlogging tolerance. *Theoretical and Applied Genetics***,** 1-11.
Zhang, Z., L. Wei, X. Zou, Y. Tao, Z. Liu & Y. Zheng (2008) Submergence-responsive MicroRNAs are potentially involved in the regulation of morphological and metabolic adaptations in maize root cells. *Annals of Botany,* 102**,** 509-519.
Zhao, M.-G., Q.-Y. Tian & W.-H. Zhang. (2007) Ethylene activates a plasma membrane Ca^{2+}-permeable channel in tobacco suspension cells. *New Phytologist,* 174**,** 507-515.
Zhou, G., E. Delhaize, M. Zhou & P. R. Ryan (2013) The barley MATE gene, HvAACT1, increases citrate efflux and Al^{3+} tolerance when expressed in wheat and barley. *Annals of Botany,* 112**,** 603-612.
Zhou, G., Q. Zhang, C. Tan, X.-q. Zhang & C. Li (2015) Development of genome-wide InDel markers and their integration with SSR, DArT and SNP markers in single barley map. *BMC Genomics,* 16**,** 1-8.
Zhou, M. 2010. Improvement of plant waterlogging tolerance. In *Waterlogging signalling and tolerance in plants,* eds. S. Mancuso & S. Shabala, 267-285. Heidelberg, Germany: Springer-Verlag.
Zhou, M. (2011) Accurate phenotyping reveals better QTL for waterlogging tolerance in barley. *Plant Breeding,* 130**,** 203-208.
Zhou, M., P. Johnson, G. Zhou, C. Li & R. Lance (2012) Quantitative trait loci for waterlogging tolerance in a barley cross of Franklin × YuYaoXiangTian Erleng and

the relationship between waterlogging and salinity tolerance. *Crop Science,* 52**,** 2082-2088.
Zhou, M. X., H. B. Li & N. J. Mendham (2007) Combining ability of waterlogging tolerance in barley. *Crop Science,* 47**,** 278-284.
Zhou, W., D. Zhao & X. Lin (1997) Effects of waterlogging on nitrogen accumulation and alleviation of waterlogging damage by application of nitrogen fertilizer and mixtalol in winter rape (*Brassica napus* L.). *Journal of Plant Growth Regulation,* 16**,** 47-53.
Zhu, M., M. Zhou, L. Shabala & S. Shabala (2016) Physiological and molecular mechanisms mediating xylem Na^+ loading in barley in the context of salinity stress tolerance. *Plant, Cell & Environment***,** n/a-n/a.
Zimmermann, S., T. Ehrhardt, G. Plesch & B. Müller-Röber (1999) Ion channels in plant signaling. *Cellular and Molecular Life Sciences CMLS,* 55**,** 183-203.
ZoBell, C. E. (1946) Studies on redox potential of marine sediments. *AAPG Bulletin,* 30**,** 477-513.